Department of Pathology
Department of Otorhinolaryngology – Head and Neck Surgery
Head and Neck Center
Helsinki University Hospital
and
Faculty of Medicine, Doctoral Programme in Clinical Research
University of Helsinki
Helsinki, Finland

MINOR SALIVARY GLAND ADENOID CYSTIC CARCINOMA:

DIAGNOSTIC AND PROGNOSTIC FACTORS AND TREATMENT OUTCOME

Hanna Laine

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine, University of Helsinki, for public examination in the Oral and Dental Centre hall (3rd floor), Haartmaninkatu 1, on December 17th, 2021 at 12 noon.

Supervised by

Professor Jaana Hagström
Department of Pathology, Haartman Institute, HUSLAB
Research Programs Unit, Translational Cancer Medicine
University of Helsinki
Helsinki, Finland
and
Department of Oral Pathology and Radiology
University of Turku
Turku, Finland

Professor Antti Mäkitie
Department of Otorhinolaryngology – Head and Neck Surgery
Faculty of Medicine, University of Helsinki
and
Head and Neck Center, Helsinki University Hospital
Helsinki, Finland

Docent Leif Bäck
Department of Otorhinolaryngology – Head and Neck Surgery
Head and Neck Center, Helsinki University Hospital
and
Faculty of Medicine, University of Helsinki
Helsinki, Finland

Reviewed by

Docent Heikki Teppo
Department of Otorhinolaryngology
Kanta-Häme Central Hospital
Hämeenlinna, Finland

Docent Tero Soukka
Department of Oral and Maxillofacial Surgery
Turku University Hospital
Turku, Finland

Opponent

Professor Timo Paavonen
Department of Pathology
Faculty of Medicine and Health Technology, Tampere University
and Fimlab Laboratories

Tampere, Finland
The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations.

ISBN 978-951-51-7680-6 (pbk.)
ISBN 978-951-51-7681-3 (PDF)

Helsinki University Printing House
Helsinki 2021

To my family

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred in the text by their Roman numerals:

I Hämetoja H*, Hirvonen K*, Hagström J, Leivo I, Saarilahti K, Apajalahti S, Haglund C, Mäkitie AA*, Bäck L*. Early stage minor salivary gland adenoid cystic carcinoma has favourable prognosis. Virchows Arch. 471: 785-792, 2017.

II Hämetoja H, Hagström J, Haglund C, Bäck L, Mäkitie A, Syrjänen S. Polyomavirus JCPyV infrequently detectable in adenoid cystic carcinoma of the oral cavity and the airways. Virchows Arch. 475: 609-616, 2019.

III Hämetoja H, Mäkitie A, Bäck L, Leivo I, Haglund C, Sorsa T, Hagström J. Matrix metalloproteinase-7, -8, -9, -15, and -25 in minor salivary gland adenoid cystic carcinoma. Pathol Res Pract. 217: 153293, 2021.

IV Hämetoja H, Andersson L.C, Mäkitie A, Bäck L, Hagström J, Haglund C. Antizyme inhibitor 2 (AZIN2) associates with better prognosis of head and neck minor salivary gland adenoid cystic carcinoma. APMIS. 129: 503-511, 2021.

*Equal contribution
Study I has been published in the thesis by Karoliina Hirvonen: Adenoid cystic carcinoma of salivary glands: Diagnostic and prognostic factors and treatment outcome. Dissertationes Scolae Doctoralis ad Sanitatem Investigandam universitatis Helsinkiensis 59/2017.

ABBREVIATIONS

ACC	Adenoid cystic carcinoma
AWD	Alive with disease
AZIN	Antizyme inhibitor
BKPyV	BK polyomavirus
CEA	Carcinoembryonic antigen
CT	Computed tomography
DFS	Disease-free survival
DNA	Deoxyribonucleic acid
DOC	Dead of other cause
DOD	Dead of disease
DSS	Disease-specific survival
EBV	Epstein-Barr virus
ECM	Extracellular matrix
EMA	Epithelial membrane antigen
END	Elective neck dissection
HPV	Human papillomavirus
HPyV	Human polyomavirus
HR	High risk
JCPyV	John Cunningham polyomavirus
LR	Low risk
MaSG	Major salivary gland
MCC	Merkel cell carcinoma
MCPyV	Merkel cell polyomavirus
MiSG	Minor salivary gland
MMP	Matrix metalloproteinase
MYB	Myeloblastosis oncogene
NED	No evidence of disease
NFIB	Transcription factor nuclear factor I/B
ODC	Ornithine decarboxylase
OS	Overall survival
qPCR	Quantitative polymerase chain reaction
RT	Radiotherapy
SCC	Squamous cell carcinoma
SGC	Salivary gland cancer
SV40	Simian vacuolating virus 40
T-ag	T antigen
TNM	Tumor, node, metastasis
UICC	Union for International Cancer Control
WHO	World Health Organization

ABSTRACT

Salivary gland cancer (SGC) comprises less than 5% of head and neck malignancies. SGCs can occur in both major and minor salivary glands. Approximately 60 new cases of major SGC occur annually in Finland. Unfortunately, national statistics does not show the annual number of minor salivary gland (MiSG) SGCs. The World Health Organization (WHO) classifies 19 histologically distinct SGCs. The etiology of SGC is unknown and the risk factors include smoking, alcohol consumption, increased age, female sex, ionizing radiation, and occupational exposure, e.g. to rubber and nickel. In addition, oncoviruses might increase the risk for SGC.

Adenoid cystic carcinoma (ACC) is denoted as the second most common SGC worldwide. In Finland, ACC is the most common histological subtype according to a nationwide study. ACC is a slow-growing neoplasm and has a tendency for perineural invasion. ACC shows three distinct histological growth patterns: cribriform, tubular, and solid. Surgery is the pivotal treatment modality, but treatment is modified according to tumor site, biological aggressiveness, and stage of the disease, which is determined according to the Union for International Cancer Control (UICC) Tumor-Node-Metastasis (TNM) classification. Postoperative radiotherapy is recommended for all patients, but chemotherapy is used mainly for inoperable, recurrent, or metastatic disease. Recurrencies affect approximately 50% of patients. Especially local and distant recurrent tumors are fairly common, with distant metastasis being more frequent and lungs the most common site. ACC has a good 5-year disease-specific survival (76-88%), but the 10-year survival (34-67%) is clearly worse. Prognostic factors affecting survival are tumor site, TNM classification, histology, surgical margin status, and distant metastasis.

In this thesis, the goal was to collect all patient data and tumor samples of the MiSG ACC patients diagnosed between years 1974 and 2012 in the Helsinki University Hospital area. Patient and tumor characteristics, treatment, outcome, and their associations were studied. To evaluate the viral role in ACC samples, the presence of three polyomaviruses were assessed by the qPCR method and genotyping of 24 human papillomaviruses (HPV) was performed with a Multiplex HPV Genotyping Kit. Furthermore, by immunohistochemistry matrix metalloproteinase (MMP)-7, -8, -9, -15, and -25 and antizyme inhibitor (AZIN) 1 and 2 in ACC were studied.

In this study, the most common tumor site was the palate. Of patients, 94% were treated with curative intent. Moreover, 53% of patients suffered from recurrent ACC of which 36% were local, 12% regional, and 52% distant. Almost all distant metastases appeared within 10 years. The 5- and 10-year overall survival and disease-specific survival were 70% and 79%, and 42% and 52%, respectively. Stage I ACC patients had better survival than patients with higher stages (II-IV). Of interest, John Cunningham polyomavirus (JCPyV) DNA was

found in 10% of the tumor samples. In the immunohistochemical studies on MMPs, abundant MMP-7 and -25 were associated with better survival. High tumorous MMP-9 associated with advanced stage and high MMP-15 immunoexpression with poorer survival. Intriguingly, abundant MMP-9 immunoexpression in inflammatory cells in the vicinity of ACC and in luminal material of pseudocysts of ACC associated with better survival and fewer local recurrent tumors. Immunoexpression of AZIN2 was abundant in well-differentiated tumor tissue (cribriform and tubular), but in the solid pattern the expression was negative or mild. AZIN2 immunoexpression associated with better survival.

To conclude, these results show that especially stage II ACC should be considered as advanced disease and patients would benefit from more aggressive treatment. Follow-up time should be prolonged for at least ten years. JCPyV could participate in the pathogenesis of a small proportion of ACC. MMPs could participate in ACC carcinogenesis by tissue modulation, activating different signaling pathways, and by immunomodulation. MMP-7, -9, -15, and -25 are related to prognostic factors. High AZIN2 immunoexpression in well-differentiated ACC could be related to a functioning vesicle transport system of tumor cells that no longer exists in poorly differentiated ACC tissue. AZIN2 could be a prognostic factor for better survival of ACC patients.

SUMMARY IN FINNISH

Sylkirauhasten syövät ovat harvinaisia. Pään ja kaulan alueen syövistä ne käsittävät alle 5 %. Sylkirauhassyöpiä esiintyy suurissa ja pienissä sylkirauhasissa. Suomessa suurten sylkirauhasten syöpiä ilmaantuu vuosittain noin 60. Pienten sylkirauhasten syöpien ilmaantuvuutta ei tällä tarkkuudella tiedetä, koska näille ei ole omaa vastaavaa luokitusjärjestelmää. Maailman terveysjärjestö (WHO) luokittelee histologisesti 19 eri sylkirauhassyöpää. Sylkirauhassyöpien etiologia on tuntematon. Riskitekijöinä pidetään tupakointia, alkoholin käyttöä, korkeaa ikää, naissukupuolta, ionisoivaa säteilyä sekä tietyistä ammateista johtuvaa altistumista esimerkiksi kumille tai nikkelille. Myös onkovirukset saattavat lisätä sylkirauhassyövän riskiä.

Adenokystinen karsinooma (ACC) on toiseksi yleisin sylkirauhassyöpä maailmassa. Suomessa ACC on yleisin sylkirauhassyöpä. ACC kasvaa tyypillisesti hitaasti ja tunnusomaista on perineuraalinen invaasio. Histologisesti ACC:ssa esiintyy kolmea erilaista kasvutapaa: kribriforminen, tubulaarinen ja solidi. ACC hoidetaan kirurgisesti. Hoito räätälöidään potilaskohtaisesti kasvaimen paikan, arvioidun biologisen aggressiivisuuden sekä kasvaimen edenneisyysasteen (stage) mukaan, joka määritellään kansainvälisen luokittelun perusteella (the Union for International Cancer Control (UICC) Tumor-Node-Metastasis (TNM) classification). Kirurgian jälkeistä sädehoitoa suositellaan kaikille ACC potilaille. Kemoterapiaa tarjotaan potilaille, joiden kasvainta ei pystytä leikkaamaan sekä taudin uusiessa tai lähettäessä etäpesäkkeitä. Tautiuusiutumia esiintyy noin 50 %:lla potilaista. Tavallisimmin ACC joko uusiutuu alkuperäiselle paikalleen tai lähettää etäpesäkkeitä keuhkoihin. ACC:n tautispesifinen viisivuotisennuste on korkea (76-88 %), mutta 10-vuotisennuste (34-67 %) on alhaisempi. Selviytymistä ennustavat kasvaimen sijainti, histologinen kasvutapa, leikkausmarginaalit, levinneisyysluokitus sekä etäpesäkkeiden esiintyminen.

Väitöskirjatutkimuksessa kerättiin potilastiedot ja kasvainnäytteet pienten sylkirauhasten ACC potilailta, jotka oli hoidettu vuosien 1974-2012 aikana Helsingin yliopistollisessa keskussairaalassa. Näistä tutkittiin potilas -ja kasvainkohtaiset tunnusmerkit, hoitokäytännöt, selviytyminen sekä näiden väliset mahdolliset yhteydet. qPCR-menetelmällä kasvainnäytteistä määritettiin kolmen polyoomavirustyypin esiintyminen ja lisäksi tehtiin 24:n ihmisen papilloomaviruksen tyypitys. Immunohistokemiallisina värjäyksinä tehtiin matriksin metalloproteinaasi (MMP) -7, -8, -9, -15 -ja 25 ja antizyme estäjät (AZIN) 1 ja 2.

Pienten sylkirauhasten ACC esiintyi yleisimmin suulaessa. Potilaista 94 % hoidettiin kuratiivisesti, joista 53 %:lla ACC uusiutui. 36 %:ia tautiuusiutumista oli paikallisia ja 12 %:ia sijaitsi kaulalla. 52 %:lle potilaista tuli etäpesäkkeitä, jotka ilmestyivät valtaosin 10 vuoden aikana taudin

diagnosoimisesta. Viisi ja kymmenen vuotinen kokonaisselviytyminen ja tautikohtainen selviytyminen olivat 70 ja 79 % sekä 42 ja 52 %. Stage I ACC potilaat selviytyivät paremmin verrattuna stage II-IV-potilaisiin. John Cunningham polyoomavirusta (JCPyV) esiintyi 10 %:ssa ACC-näytteitä. Immunohistokemiallisissa tutkimuksissa runsas MMP-7 ja -25 immunovärjäytyvyys yhdistyivät parempaan selviytymiseen. Runsas MMP-9 värjääntyvyys yhdistyi taudin levinneisyyteen ja MMP-15 heikompaan selviytymiseen. Lisäksi havaittiin tulehdussolujen ja ACC:n luminaalisten pseudokysta-alueiden runsaan MMP-9 immunovärjäytymisen yhdistyvän sekä parempaan selviytymiseen että vähäisempään määrään paikallisia uusiutumia. AZIN2 immunovärjäytyvyys oli puolestaan yleisempää hyvin erilaistuneissa kasvaimissa (kribriforminen ja tubulaarinen kasvutapa) verrattuna huonosti erilaistuneisiin (solidi kasvutapa) kasvaimiin. Runsas AZIN2 immunovärjäytyvyys yhdistyi lisäksi parempaan ennusteeseen.

Nämä tulokset osoittavat, että erityisesti stage II ACC:tä on pidettävä pidemmälle edenneenä kasvaimena ja potilaat voivat hyötyä tehokkaammasta hoidosta. ACC:n kliinisen seurannan tulisi olla ainakin 10 vuotta, jotta mahdollisimman monet etäpesäkkeet havaittaisiin nopeasti. Tutkimus antoi viitteitä, että JCPyV mahdollisesti osallistuu ACC:n kehittymiseen. Myös MMP:t voivat osallistua ACC:n syntyyn muokkaamalla kudoksia, aktivoimalla signaalireittejä sekä muokkaamalla paikallista puolustusvastetta. MMP-7, -9, -15 ja -25 olivat yhteydessä ACC:n ennusteeseen. Runsas AZIN2 värjäytyvyys hyvin erilaistuneessa ACC:ssa voi liittyä sen osallisuuteen kasvainsolun toimivassa vesikkelien kuljetussysteemissä, joka ei mahdollisesti toimi enää huonosti erilaistuneessa kasvaimessa. AZIN2 värjääntyvyyttä voisi siten käyttää ennustemerkkinä ACC-potilaiden paremmasta selviytymisestä.

1. INTRODUCTION

Infrequent salivary gland cancers (SGC) form a heterogeneous group comprising 19 histological subtypes [1]. SGCs can occur in both major (MaSG) and minor (MiSG) salivary glands. MiSGs are small glandular structures located in the oral cavity and oropharynx. Similar small excreting glands are distributed in the aerodigestive tract and in the ears. In this thesis study, all minor salivary, mucous, and ceruminous glands in the head and neck area were included into the category of MiSGs. Tumors occurring in MiSGs are malignant in 40-90% of cases and most often, epithelial in origin [1]. Hence, here, these malignancies are called SGCs.

Adenoid cystic carcinoma (ACC) is the second most common SGC worldwide, but in a few nationwide studies, including Finland, ACC is denoted as the most common SGC [1-4]. ACC is reported as the most common MiSG malignancy, frequently seen in the palate [2, 3, 5-7].

ACC is diagnosed often at 50 years of age and the disease shows a female predilection [6, 8]. Clinically, ACC is described as an unpredictable high-grade neoplasm with a tendency to produce both local and distant metastases even after a long follow-up [7, 8]. Treatment is surgical with or without radiotherapy, and chemotherapy is usually used in advanced, recurrent, or metastatic diseases [8, 9]. Prognostic factors include the Union for International Cancer Control (UICC) Tumor-Node-Metastasis (TNM) classification, tumor location, histology, surgical margins, perineural invasion, and recurrencies [8, 10, 11]. For ACC, 5- and 10-year disease-specific survival (DSS) rates are 76-88% and 34-67%, respectively [8, 11].

Etiology of SGC or ACC is not well-known. Risk factors for SGC include ionizing radiation [12], industrial exposure to rubber or nickel [13], and previous primary cancer [14, 15]. A recently described ACC-specific oncogenic event is gene translocation of *MYB*, resulting in a fusion of the myeloblastosis (*MYB*) oncogene to the transcription factor nuclear factor I/B (*NFIB*) [16]. Although the fusion gene does not seem to determinate prognosis [17], it could be used as a biomarker in diagnostics [18].

This study aimed to investigate the clinical presentation, treatment, and outcome of MiSG ACC patients in the Helsinki University Hospital district. Moreover, the aim was to assess the presence of human polyomaviruses (HPyV) and human papillomaviruses (HPV) in ACC in order to examine the role of viruses in the carcinogenesis of ACC. Matrix metalloproteinases (MMP) and antizyme inhibitors (AZIN) were investigated as prognostic markers in ACC.

2. REVIEW OF THE LITERATURE

2.1 SALIVARY GLANDS

Salivary glands are divided into MaSGs and MiSGs. MaSGs consist of three paired glands, namely the parotid gland, submandibular gland, and sublingual gland. Small glandular structures beneath the epithelium in the head and neck area are called minor salivary and mucous glands, MiSGs. Recently, Valstar et al. have reckoned to discover a new pair of salivary glands, the tubarial salivary glands, in the nasopharynx [19].

2.2 ANATOMY AND MORPHOLOGY OF MINOR SALIVARY GLANDS

Between 450 and 1000 MiSGs are distributed in the aerodigestive tract (oral cavity, oropharynx, nose, paranasal sinuses, pharynx, larynx, and trachea) [20]. Th bronchi and esophagus have the similar small glands. MiSGs, sized 1-5 mm, are unencapsulated and located beneath the surface epithelium. MiSGs are responsible for production and secretion of mucous and seromucous saliva that aid mastication, deglutition, digestion, and further protects teeth and mucosa. [21]

Small glandular structures in the external auditory canal are called ceruminous glands; these are located beneath the skin. The number of ceruminous glands varies between 1000 and 2000. These modified apocrine glands produce cerumen, which protects the ear from physical damage and microbial invasion. [22]

The acinar-ductal unit is the basic structure of all salivary and similar glands, and it is composed of epithelial and myoepithelial cells (Figures 1 and 2). Acinus is formed by small, pear-shaped groups that are surrounded by basement membrane. Acinus cells are serous or mucous. Serous cells are pyramid-shaped with cytoplasm filled with zymogen granules. Their nuclei are basally oriented and the cytoplasm stains basophilic. Mucinous cells consist of a round, basal nucleus and cytoplasm with vacuoles containing mucin. At the periphery of the acini, there are myoepithelial cells capable of contraction. Acini form several secretory units that open via short ducts directly through the mucosa. [21] The ductal system of MiSG is minimalistic with shorter tracts than in the MaSG [23].

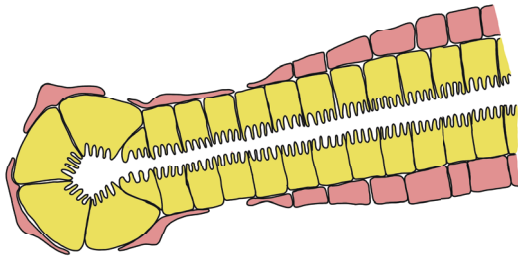

Figure 1. Acinar-ductal unit of the salivary gland. Epithelial cells appear in yellow and myoepithelial cells in pink. Courtesy of Ruusu Hulmi.

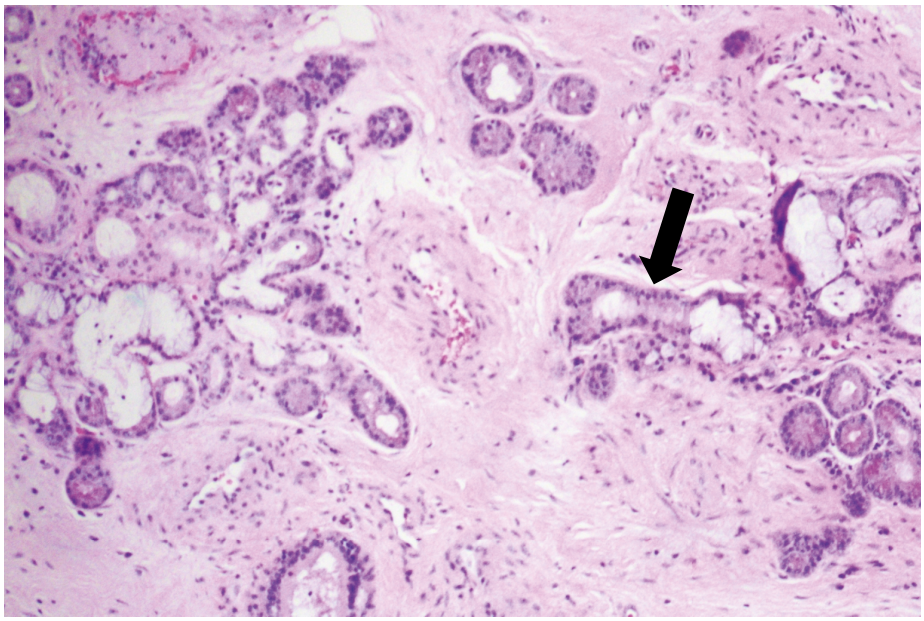

Figure 2. Acinar-ductal unit (arrow) of the mucous gland in the sinonasal cavity. Magnification x 10. Courtesy of Professor Jaana Hagström.

2.3 EPIDEMIOLOGY OF SALIVARY GLAND CANCER (SGC) AND ADENOID CYSTIC CARCINOMA (ACC)

Annual incidence of epithelial malignancies of major and minor salivary glands varies from 0.3 to 3 per 100 000 [24]. SGC represents 0.5% of all malignancies and 3% to 5% of all head and neck malignancies worldwide [25, 26].

Malignancies of MiSGs account for 10% to 20% of all SGCs [20, 26]. Approximately 60 new cases of MaSG SGCs are diagnosed in Finland annually. According to the Finnish Cancer Registry, the age-adjusted incidence rates per 100 000 person-years between years in 2015-2019 were 1.5 for females and 1.33 for males with MaSG SGC (www.cancer.fi). Due to the lack of TNM classification exclusively for MiSG malignancies, the incidence rates for MiSG SGCs are not available from the Finnish Cancer Registry.
ACC represents less than 1% of head and neck malignancies and 10% of all salivary gland neoplasms. Approximately 30% of minor SGCs are ACC. [27, 28] ACC is the second most common SGC worldwide after mucoepidermoid carcinoma [1], but in nationwide studies from Finland and Denmark ACC has been shown to be the most common SGC subtype [2, 3]. According to location, ACC is the most common entity of SGCs in the sinonasal cavities, larynx, trachea [10], and external ear canal [21]. ACC of oral cavity and oropharynx is reported to be the most common or the second most common SGC subtype [7, 10]. The Finnish Cancer Registry does not directly report the annual number of new cases of MiSG SGCs or ACCs. A medical record system search found three MiSG ACC diagnoses in 2018 in the Helsinki University Hospital district.

2.4 RISK FACTORS FOR SGC

Risk factors for SGC are largely unknown. Specific risk factors for ACC have not been recognized. Smoking, alcohol consumption, gender, and aging are cancer risk factors in general and these are also related to SGC [13, 29].

Based on studies of atomic bomb survivors, ionizing radiation is a well-known risk factor for SGC [12]. Heavy doses of ionizing radiation to the head and neck area increase the prevalence of oral and oropharyngeal adenocarcinomas rather than squamous cell carcinomas (SCCs) [12]. In addition, cervicofacial radiotherapy has been shown to be a risk factor for SGC [14].

According to an epidemiological study, several occupations and industries are potential risk factors for SGC [14]. Especially workers in rubber or nickel-using industries have an increased risk for SGC [13].

Previous primary cancer increases the risk for SGC [14, 15]. Falchook et al. discovered that any previous cancer elevates the risk for a second primary SGC, particularly among women [15].

Oncoviruses as etiological factors have been studied in SGC with varying results. Studies concluding against a viral etiology are more numerous than studies supporting oncoviruses as etiological factors. HPVs, such as HPV16 and 18, human herpes virus 8, and Epstein-Barr virus (EBV) have been shown not to participate in the etiopathogenesis of SGC [30-32]. However, Hühns et al. have provided a weak evidence that HPV infection could be part of the salivary gland tumor etiopathogenesis [32]. EBV infection has been shown to be associated with a rare SGC, namely lymphoepithelial carcinoma, in certain

populations of indigenous people. In the Western population, the association is infrequent. [1]

2.5 MOLECULAR PATHOGENESIS OF ACC

The molecular pathogenesis of ACC is not yet well-recognized. Recently, the key oncogenic event of ACC has been described to be the gene translocation of *MYB* t(6;9)(q21-24;p13-23) [16]. This translocation results in a fusion of the myeloblastosis (*MYB*) oncogene to the transcription factor nuclear factor I/B (*NFIB*) [16]. It affects at least 50% of ACC tumors [33]. The activity of *MYB-NFIB* fusion gene leads to elevated levels of *MYB* transcript and overexpression of the protein Myb [33]. *MYB* is frequently overexpressed in ACC with *MYB-NFIB* fusion gene, although the overexpression is detected as well in fusion-negative ACC [34]. In addition, ACC shows more rarely a t(8;9) translocation in which *MYBL1* oncogene and transcription factor gene *NFIB* form a fusion [1]. *MYB/MYBL1* fusions have not been found in other salivary gland tumors and can be used as a biomarker for facilitating diagnostics [34-37]. According to a recent meta-analysis, *(t6;9) (MYB-NFIB)* does not determine prognosis [17].

The Notch pathway alterations have been identified in ACC [1, 38, 39]. Chen et al. have shown that *NOTCH1* knock-down decreases the growth and migration of ACC cells *in vitro* and metastatic potential *in vivo* [40]. *NOTCH1* alterations are associated with the behavior of ACC, and this mutation type defines a subgroup of ACCs with more solid histology, liver and bone metastases, and otherwise poor prognosis [41].

A transmembrane tyrosine kinase receptor c-kit protein (cluster of differentiation 117 [CD117]) is a growth factor expressed by the luminal epithelial cells of ACC. c-kit expression has been detected in 90% of ACC tumors, which have been shown to be associated with high-grade tumors. [42] Myoepithelial cells of ACC express extensively an epidermal growth factor receptor (EGFR). High expression levels of EGFR are associated with advanced histological grade [43]. The mechanisms of *c-kit* and *EGFR* in the pathogenesis of ACC is not fully understood. They are frequently overexpressed in ACC but infrequently mutated or amplified [1].

2.6 DIAGNOSIS OF ACC

2.6.1 SYMPTOMS

Presenting symptoms in MiSG and MaSG SGCs are largely similar. According to the review article by Coca-Pelaz et al. the most common

symptoms are slowly growing mass and pain [7]. Symptoms may vary due to site and tumor size. ACC occurring in the palate might present a mass resembling a fibroma, ulceration, or even an oro-antral fistula to the maxillary sinus [7]. Initial symptoms in ACC of paranasal sinuses are described to be pain, unilaterally blocked nose, and repeated epistaxis [44]. Studies concerning MiSGs of the oral cavity, oropharynx, and upper respiratory tract list symptoms such as lump, pain, ill-fitting denture, salivary duct blockage, ulceration, and delayed healing [45, 46]. Detection of regional metastasis or metastatic disease at the time of initial diagnosis is uncommon. Shum et al. reported 3.6% cervical and 7.1% distant metastasis at the diagnosis of MiSG ACC [46].

2.6.2 HISTOLOGICAL DIAGNOSIS

At the cellular level, ACC tissue consists of epithelial and myoepithelial cells. The tumor cells are small, cuboidal, and basophilic with hyperchromatic nuclei and scant cytoplasm [1, 21].

WHO classification (2017) divides SGCs into 19 different histological subtypes (Table 1). ACC is graded as the second most common histological subtype. [1] Histologically, ACC tissue presents different growth patterns, namely cribriform, tubular, and solid (Figures 3 and 4). [1, 47] A single tumor can present various growth patterns, but the richest pattern determines the classification. Cribriform and tubular growth patterns are more frequent than solid. A tumor that consists of more than one-third of solid type shows aggressive behavior. [47]

Table 1. World Health Organization (WHO) classification of malignant salivary gland tumors according to WHO/IARC, 4th edition [1].

Mucoepidermoid carcinoma
Adenoid cystic carcinoma
Acinic cell carcinoma
Polymorphous adenocarcinoma
Clear cell carcinoma
Basal cell adenocarcinoma
Intraductal carcinoma
Adenocarcinoma, not otherwise specified
Salivary duct carcinoma
Myoepithelial carcinoma
Epithelial-myoepithelial carcinoma
Carcinoma ex pleomorphic adenoma
Secretory carcinoma
Sebaceous adenocarcinoma
Carcinosarcoma
Poorly differentiated carcinoma
Undifferentiated carcinoma

	Large cell neuroendocrine carcinoma
	Small cell neuroendocrine carcinoma
Lymphoepithelial carcinoma	
Squamous cell carcinoma	
Oncocytic carcinoma	
Uncertain malignant potential	
Sialoblastoma	

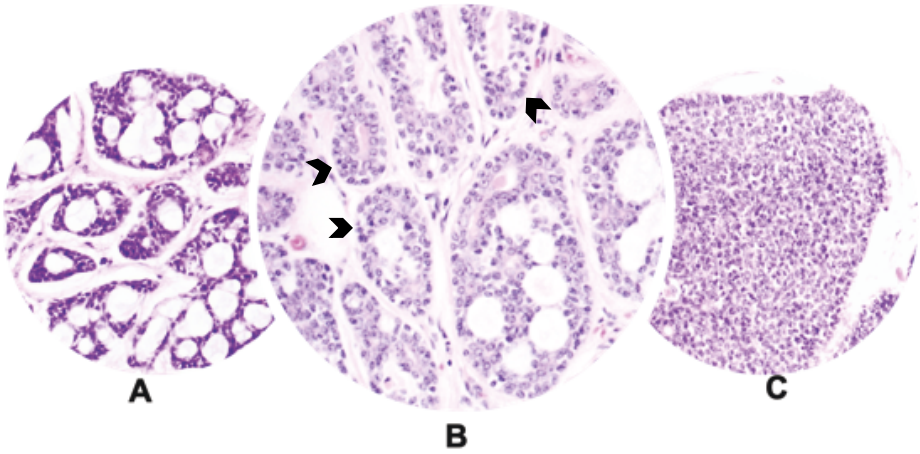

Figure 3. Growth patterns of adenoid cystic carcinoma. A: Cribriform. B: Tubular (arrowhead) and cribriform. C: Solid. Magnification x 20. Courtesy of Professor Jaana Hagström.

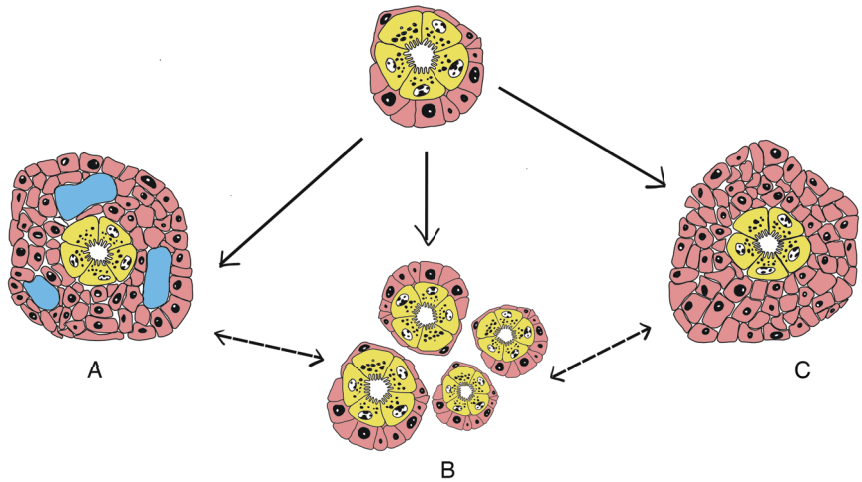

Figure 4. Emulation of a ducto-acinar unit of salivary gland (above) in different growth patterns of adenoid cystic carcinoma. Epithelial cells appear in yellow and myoepithelial cells in pink. A: In the cribriform growth pattern, epithelial cells form ductal structures surrounded by myoepithelial cells and pseudocysts containing glycosaminoglycans and basal lamina. B: Tubular structures are formed when inner epithelial cells are surrounded by a single to a few layers of myoepithelial cells. C: The solid growth pattern is formed with the proliferation of neoplastic myoepithelial cells along with a few ductal structures. [47] Courtesy of Ruusu Hulmi.

From a representative biopsy sample, a pathologist is able to diagnose the subclassification of the SGC and evaluate the histological grade. From a histological perspective, several SGCs share similar features. To determine the correct diagnosis, it is important to see the tumor surroundings and to perform immunohistochemical staining. Immunohistochemical markers that aid in differentiating between epithelial and myoepithelial cells have been used in ACC diagnostics. These are CD117, p63, and smooth muscle actin (SMA) [1]. CD117 shows the presence of inner epithelial tumor cells, and p63 and SMA stain peripheral myoepithelial tumor cells [1, 48, 49]. Epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) do not stain pseudocysts of ACC but are positive in the true glands of ACC [50]. New tools for challenges in diagnostics are MYB immunohistochemistry and MYB break-apart fluorescent *in situ* hybridization (FISH) [36].

In case of ACC, differential diagnoses to exclude are pleomorphic adenoma, polymorphous adenocarcinoma (PAC), secretory carcinoma (SC), epithelial-myoepithelial carcinoma (EMC) and basal cell adenocarcinoma (BAC). PAC tissue is immunohistochemically positive for cytokeratin-7 (CK7), S-100 protein, CEA, mammaglobin, and basal and myoepithelial cell marker p63. In addition, ACC usually has a higher Ki-67 proliferation index. SC originates from one cell type. SC immunoexpress mammaglobin, vimentin, and S-100. EMC has a biphasic nature, as does ACC; the inner luminal epithelial cells express CK7 and the outer myoepithelial cells p63, SMA, and calponin. Collagen type IV staining is positive in the surrounding stroma of basement membrane material. Ki-67 shows variable expression. BAC shows dual-cell composition, whereas CK7 stains epithelial cells and SMA immunoexpression is seen in myoepithelial cells. Compared with basal cell adenoma, BAC shows invasion. Compared with ACC, BAC shows more vesicular nuclei, peripheral palisading, and more squamous and sebaceous parts. [1, 37]

2.6.3 PREOPERATIVE INVESTIGATIONS

Preoperative imaging options for patients with suspected or confirmed MiSG malignancy are computed tomography (CT) and magnetic resonance imaging (MRI). CT and MRI show the exact location and size of the tumor in

relation to major neurovascular structures, perineural and skull base invasion, and intracranial extension [51]. CT is used to delineate bone invasion. MRI reveals better soft tissue extensions such as neural or soft tissue invasion, diffuse growth patterns, and lymphadenopathy [7]. Imaging should also provide accurate staging of the regional lymph nodes and evaluation of distant metastases [51]. In addition, fine-needle aspiration could be used for assessing the diagnosis although this technique is not widely used for MiSG tumors.

2.6.4 STAGING

The stage of the disease depending on tumor size, lymph node involvement, and distant metastasis is determined according to UICC TNM classification [52]. Currently, TNM classification is exclusively used for malignancies of MaSGs. Due to the lack of a specific staging system, the classification of MiSG malignancies is performed according to the corresponding classification of the head and neck SCC in the same location. Table 2 shows TNM classification of malignant tumors in the lip and oral cavity.

Table 2. Tumor-Node-Metastasis (TNM) classification of malignant tumors in the lip and oral cavity according to the Union for International Cancer Control, 8th edition [52].

T-Primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension and 5 mm or less in depth of invasion
T2	Tumor 2 cm or less in greatest dimension and more than 5 mm but no more than 10mm in depth of invasion or tumor more than 2cm but not more than 4cm in greatest dimension and depth of invasion no more than 10mm
T3	Tumor more than 4 cm in greatest dimension or more than 10mm in depth of invasion
T4a	(Lip) Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin (of the chin or the nose)
T4a	(Oral cavity) Tumor invades through the cortical bone of the mandible or maxillary sinus, or invades the skin of the face

T4b	(Lip and oral cavity) Tumor invades masticator space, pterygoid plates, or skull base, encases internal carotid artery
N-Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension without extranodal extension
N2	Metastasis described as:
	N2a: Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension, without extranodal extension N2b: Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension, without extranodal extension N2c: Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension, without extranodal extension
N3a	Metastasis in a lymph node more than 6 cm in greatest dimension without extranodal extension
N3b	Metastasis in a single or multiple lymph nodes with clinical extranodal extension
M-Distant metastasis	
M0	No distant metastasis
M1	Distant metastasis

2.7 TREATMENT

The principal treatment for all SGCs is surgery with or without radiotherapy (RT) [9, 20, 26, 51, 53, 54]. For MiSG cancer, the treatment strategy is customized depending on the site, extent of disease, histological grade, and biological behavior [20].

2.7.1 TREATMENT OF THE PRIMARY SITE

The goal of the surgical approach is to ensure adequate tumor-free margins. However, the concept of adequate free margins is not well-established in MiSGs. Especially MiSG ACC often infiltrates into adjacent tissues and achieving free margins might be challenging. Intraoperative pathologic examination, i.e. frozen section, supports the surgeon since a frozen section study is shown to be a reliable aid in intraoperative decision-making. [9] The surgical approach varies from open to endoscopic depending on the location. In the oral cavity, for a localized low-grade tumor a wide excision might be the only treatment modality. For advanced tumors radical surgery (mandibulectomy or maxillectomy) with postoperative RT is the treatment of choice. [20, 51] Laryngeal ACC is often diagnosed as advanced and non-operable. Definite RT is a treatment alternative for a total laryngectomy [55].

2.7.2 TREATMENT OF THE NECK

Surgical treatment of the neck is planned individually based on the disease and most often neck dissection is selective. Among minor SGC patients, 15% have a clinical or radiologic sign of neck metastasis (N+) [20]. In ACC, Amit et al. have observed 16% of patients to have N+ neck at presentation, and the total proportion of histologically confirmed neck metastasis was as high as 29% [56]. In the primary treatment of ACC, RT is seldom used as a sole treatment modality for the neck. Surgical treatment of the neck combined with RT has a significantly better survival outcome than RT alone [57].

Treatment of patients without clinically evident neck metastasis (N0) is controversial. Recent guidelines recommend offering elective neck dissection (END) to SGC patients with T3 and T4 tumors and high-grade malignancies [9]. END is not routinely performed in ACC. Xiao et al. have shown an association between END and longer overall survival (OS) of patients with advanced ACC [58]. Occult neck metastases have been shown in 15-44% of ACC patients [56, 59] which might support END decision. In addition, ACC in the oral cavity and oropharynx seems to produce more occult neck metastasis than MaSG ACC [56, 59].

2.7.3 RADIOTHERAPY

A recent guideline recommends postoperative RT to all patients with ACC [9]. Postoperative RT is an effective method to prevent local recurrent tumors among MiSG ACC patients [60, 61]. RT is suggested for high-grade and advanced tumors, positive margins, perineural, vascular, lymphatic, or bone invasion, lymph node metastasis, recurrent tumors, and such locations such as

sinonasal cavities, oropharynx, and floor of mouth [9, 51]. The optimal treatment approach is a dose of 60 Gy in 30 fractions for most MiSG cancer patients [60]. Primary RT is used for patients with medically or technically inoperable tumors and for palliative treatment [62, 63]. Definite proton beam therapy could be used as an alternative treatment modality since it is shown to give promising local control for patients with inoperable ACC [64].

2.7.4 CHEMOTHERAPY

The role of chemotherapy in ACC is generally restricted to inoperable or recurrent tumors and metastasis [65, 66]. Even though the response rate to chemotherapy is low, this treatment can relieve symptoms of ACC patients [67]. The studies on chemotherapy agents usually have small numbers of participants, and the response rates to chemotherapy agents have been modest [65, 66]. For salivary gland ACC, the first-line chemotherapy options are mitoxantrone, vinorelbine, or epirubicin, but neither paclitaxel nor cisplatin is recommended. For combination chemotherapy, the available studies suggest cisplatin and anthracycline, although the combination has more toxic effects than single-agent chemotherapy. [66]

2.8 PROGNOSIS AND PROGNOSTIC FACTORS

The prognosis of ACC is generally considered disheartening due to the poor long-term survival rates. Five-year survival is usually high, whereas the 10-year survival curve drops markedly. For MiSG ACC, depending on the study, 5- and 10-year OS rates are 62-92% and 54-72%, respectively [44, 45, 68, 69]. The corresponding 5- and 10-year DSS rates are 43-76% and 53-74%, respectively [44, 70, 71].

Studies on MiSG ACC have shown that independent prognostic factors for OS, DSS, and disease-free survival (DFS) are T class [45, 68, 70], N class [68, 70], stage [44, 68], margin status [68, 70, 72], and solid growth pattern [44].

Overall, prognostic factors for both MiSG and MaSG ACC are similar. High T and N classes, advanced stage, tumor-positive surgical margins, perineural invasion, solid growth pattern, aging, and gender are independently related to worse survival [44, 45, 68, 70, 72-77]. Li et al. showed that MiSG ACC patients with neck metastasis at presentation had increased a risk of death compared with MaSG ACC patients [78]. To gain optimal local control for ACC, radical surgical resection with tumor-free margins is crucial [79]. According to Bianchi et al., positive surgical margins and T3-4 tumors were associated with worse locoregional control [70]. Sufficient postoperative RT was an independent prognostic factor for better OS and local control, whereas chemotherapy did not improve prognosis [73, 74]. Luksic et al. showed that perineural invasion

decreases DSS [71]. Solid ACC has been shown to associate significantly with lower OS [44, 72]. In the solid pattern, tumor cells are highly proliferative, which partly explains the aggressive behavior. High Ki-67 immunoexpression has been revealed to be associated with worse survival of SGC patients [80]. In ACC, high Ki-67 immunoexpression is shown to be associated with poorer OS, large tumor size, and recurrencies [81, 82].

ACC has a propensity for distant metastasis after a prolonged period, which is related to declining long-term survival. According to previous studies, 31-38% of ACC patients developed distant metastasis [79, 83]. The vast majority of distant metastasis occur in the lungs, followed by bone, liver, and brain [79, 83]. Patients with lung metastasis have a longer survival time than patients with other distant metastasis sites [83, 84]. Distant metastasis rates are high among MiSG ACCs of the maxillary sinus and tongue [83]. In addition, solid growth pattern [83] and positive margins [74] predict the occurrence of distant metastasis.

2.9 HUMAN POLYOMAVIRUSES (HPYV)

Thus far, the human polyomavirus (HPyV) family comprises 13 members [85]. These are non-enveloped DNA viruses with circular double-stranded genome of size varying from 5100 to 5400 base pairs (bp) (Figure 5) [86].

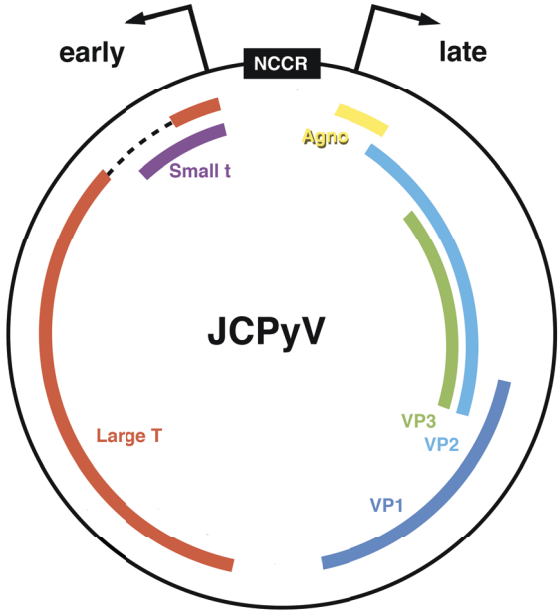

Figure 5. Genome of the John Cunningham polyomavirus, 5100 bp. The genome consists of a noncoding control region (NCCR) and two transcriptional units, namely the early and late regions. Large T antigen and small t antigen are encoded by the early gene region, whereas the late region encodes viral capsid proteins VP1, VP2, and VP3. [86] Courtesy of Ruusu Hulmi.

BK polyomavirus (BKPyV) and John Cunningham polyomavirus (JCPyV) were the first HPyVs identified in the 1970s [87, 88]. They can cause serious harm to immunocompromised patients. BKPyV infection could lead to polyomavirus-associated nephropathy in renal transplant patients. JCPyV has a causative role in a fatal central nervous system demyelinating disease, progressive multifocal leukoencephalopathy. In the adult population, the seroprevalence of BKPyV and JCPyV are 82-99% and 39-81%, respectively [89]. However, the primary exposure to these viruses is likely to occur in childhood, as the seroprevalence in children under 21 years of age is similar to that of the adult population [90].
Simian vacuolating virus 40 (SV40) is not a HPyV but a monkey polyomavirus that was inoculated in humans via the SV40-contaminated polio vaccine in the 1950s until the mid-1960s. Although this mode of SV40 transmission is no longer possible, the estimated SV40 seroprevalence is 2%, in both adults and children [90]. SV40 is capable of causing cancer in animal models [91].

2.9.1 HPYVS IN CANCER

According to an estimation by de Martel et al., in 2018 oncogenic viruses globally caused 8.3% of human cancers [92]. The International Agency for Research on Cancer (IARC) has classified seven viruses (EBV, hepatitis B virus, hepatitis C virus, Kaposi´s sarcoma herpes virus or human herpes virus 8, human immunodeficiency virus type-1, human T-cell lymphotropic virus type 1, and HPV) as group 1 human carcinogens (carcinogenic to humans). It is noteworthy that, IARC has categorized JCPyV and BKPyV as grade 2B carcinogens (possibly carcinogenic to humans) and Merkel cell polyomavirus (MCPyV) as a group 2A carcinogen (probably carcinogenic to humans) due to its association with the development of Merkel cell carcinoma (MCC). Large T-ag of HPyV has properties of an oncoprotein because it can interfere with tumor suppressor proteins p53 and pRb [93]. This oncogenic transformation is mediated by T-ag when its expression is uncoupled from the later steps of the viral life cycle: viral DNA (deoxyribonucleic acid) replication, late gene expression, virion assembly, and host cell lysis. Consequently, T-ag inactivates signal transduction pathways and tumor suppressor proteins pRb and p53 leading to neoplastic formation. [89, 94]

2.9.2 HPYVS IN HEAD AND NECK CANCER

Previous studies concerning head and neck SCC have detected BKPyV in oropharyngeal SCC [95] and JCPyV in tongue, pharyngeal, and laryngeal SCC [96, 97]. Especially in oropharyngeal SCC, Carpén et al. have recently detected BKPyV in 30%, JCPyV in 14%, and SV40 in 0.6% of the tumor samples [98]. In addition, BKPyV, JCPyV, MCPyV, human polyomavirus 6 (HPyV6), human polyomavirus 7 (HPyV7), and trichodysplasia spinulosa polyomavirus (TSPyV) might stay latent in tonsillar tissue [99-102]. However, evidence is still lacking for the role of HPyVs in tonsillar tumorigenesis and whether they act alone or as a cofactors with HPV [103].

MCC is a neuroendocrine cutaneous neoplasm that occurs often in elderly individuals in the head and neck area. MCPyV is detected in 80% of MCC and is confirmed as its etiological factor [104, 105]. Whether MCPyV-positive or -negative MCC has an effect on prognosis remains unclear. However, a few studies have shown that patients with MCPyV DNA-positive MCC have a better prognosis [105-107].

The causative role of HPyVs in salivary gland tumors is unclear due to the small number of studies on the topic. Chen et al. studied the presence of HPyVs in 79 benign and five malignant salivary gland tumors [108]. In their study, MCPyV was the most frequently detected, followed by BKPyV, JCPyV, SV40, human washington university polyoma virus (WUV), HPyV6, and HPyV7. Only MCPyV and HPyV6 were detected in malignant tumors (60%). From pleomorphic adenoma and warthin´s tumor tissue, HPyVs were detected in 27% and 50%, respectively. [108]

In 1953, SV40 was shown to promote SGC formation when inoculated into new born mice [109]. At the time, this new virus was soon verified to have tumorigenic properties [110]. In addition, Dawe et al. have shown in the 1980s that polyomavirus injection could start a formation of salivary gland tumor resembling plemorphic adenoma [111]. A few studies have shown the varying presence of SV40 (2-62%) in pleomorphic adenoma [108, 112]. Nowadays, SV40 does not seem to be prevalent in normal tissues or in malignancies. In the modern era, the great importance of SV40 to humans is probably the ability to use it in transgenic cancer models [91].

2.10 HUMAN PAPILLOMAVIRUSES (HPV)

HPVs are small, nonenveloped double-stranded DNA viruses (Figure 6). Currently, over 200 HPV genotypes have been identified. HPVs belonging to alpha genera infect mucosal epithelia. These HPVs can further be categorized as low- (LR) or high-risk (HR) HPV genotypes based on their associated risk of malignancy. The following HPV genotypes are included in the list of HR-

HPVs: HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73, and -82. [113]

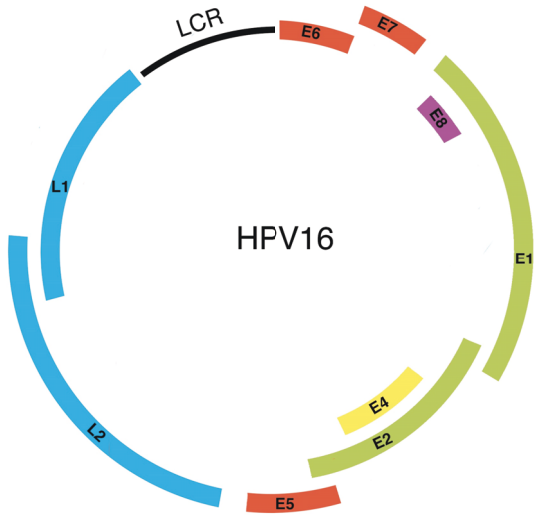

Figure 6. Structure of human papillomavirus 16 genome, 7905 bp. The genome consist of three major regions: upstream the long control region (LCR), the early (E) gene region, and the late (L) gene region. [113] Courtesy of Ruusu Hulmi.

2.10.1 HPVS IN CANCER

In 2018, 834 860 new head and neck SCC cases were registered worldwide (GLOBOCAN Registry on Cancers, www-dep.iarc.fr). According to a large meta-analysis, 31.5% of head and neck SCCs are HPV positive [114]. However, the prevalence of HPV-attributable head and neck SCCs varies globally and according to anatomic site. In addition, smoking, alcohol consumption, and gender have an impact on differences between HPV-positive and HPV-negative SCCs. [114] To present comparative figures, HR-HPVs are the main risk for cervical carcinoma of uteri, causing 80% of these carcinomas [92].

In the carcinogenesis of HPV-attributable cancers, E6 and E7 are the main oncoproteins. In brief, by disrupting cellular tumor suppressor pathways p53 and pRb, E6 and E7, respectively, alter the fundamental cellular events such as cell cycle, apoptosis, differentiation, senescence, cell polarity, and activation of immune response-related pathways. [115]

Studies have shown that HR-HPVs do not have a role in SGCs such as acinic cell carcinoma, ACC, mucoepidermoid carcinoma, epithelial-myoepithelial carcinoma, myoepithelial carcinoma, basal cell adenocarcinoma oncocytic

carcinoma, secretory carcinoma, or salivary duct carcinoma [30, 31, 116]. Interestingly, recently HR-HPV33 has been found to be related to multiphenotypic sinonasal carcinoma, a non-keratinizing SCC that is described to have features of both SCC and ACC [117].

2.11 MATRIX METALLOPROTEINASES (MMP)

MMPs form a zinc-dependent endopeptidase group that has 28 structurally related but genetically distinct members, namely collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT) MMPs, and other MMPs [118, 119]. Activated cells express MMPs [118] which process extracellular matrix (ECM) mainly composed of collagens [120]. In addition, MMPs process non-matrix bioactive molecules such as cytokines, hormones, defensins, immune mediators, other proteases, adhesion molecules, growth factors, and cell surface receptors [119]. Thus, in normal conditions, MMPs participate in physiological processes such as tissue development and remodeling and wound healing [121]. Furthermore, MMPs act in tissue destructive diseases involving ECM disruption, e.g. vascular disease, bone disorders, neurodegenerative disease, and invasion and metastasis of cancer tissue [120].

2.11.1 MMP-7 OR MATRILYSIN-1

MMP-7 is the smallest MMP and it is expressed by exocrine and mucosal epithelial cells, e.g. in salivary glands, skin, breast, intestine, pancreas, and liver. Moreover, fibroblasts and neoplastic cells of epithelial origin are able to express MMP-7. [118, 122, 123]. Interestingly, MMP-7 expression could be induced by bacteria via certain structures such as lipopolysaccharide and flagellin [118, 124]. Cytokines and hypoxia can upregulate MMP-7. ECM components, such as fibronectin, gelatin, collagen type IV, laminin, and elastin, are among the substrates of MMP-7. Cleavage of these substrates leads to breakdown of the ECM that is important for regulation of cell migration and tissue remodeling. [118] MMP-7 could activate MMP-2, -8, and -9 by cleaving their pro-forms [125].

2.11.2 MMP-8 OR NEUTROPHIL COLLAGENASE OR COLLAGENASE-2

MMP-8 was first cloned from neutrophils that were obtained from a patient with granulocytic leukemia. However, other cell types (epithelial cells, fibroblasts, endothelial cells, monocytes, macrophages, and plasma cells) could be induced to express MMP-8. [119] Stromelysin-1 (MMP-3) and MMP-7 can activate MMP-8 by proteolytic removal of the pro-peptide. The activity of

MMP-8 is inhibited by tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-2. [126] MMP-8 functions in normal physiological conditions, such as embryogenesis, and in inflammatory condition with massive tissue destruction potential, such as periodontitis [119, 126]. Moreover, MMP-8 can prevent inflammation and cancer formation [127, 128].

2.11.3 MMP-9 OR GELATINASE B

MMP-9 was first described in neutrophils and the activation is mediated by removal of the pro-domain by serine proteases or other MMPs, and due to oxidative stress that disrupts the cysteine switch [126, 129]. MMP-9 is incapable of direct proteolysis of collagen I, but it has the ability to degrade type IV collagen of basement membranes [126, 130]. Immune and inflammatory cells, such as neutrophils, lymphocytes, and dendritic cells, need MMP-9 for migration. Lemjabbar et al. have demonstrated that MMP-9 knock-out mice cannot recruit these immune cells normally after antigen presentation. [131] Both gelatinases MMP-2 and MMP-9 have been shown to participate in pulmonary diseases such as asthma and chronic obstructive pulmonary disease [126]. Itoh et al. first observed decreased tumor angiogenesis and progression in MMP-2 knock-out mice [132]. This observation has led to a wide interest in elucidating the role of MMP-2 and MMP-9 in tumorigenesis [132]. Currently, MMP-9, especially when activated, is agreed to be an important enzyme for tumor invasion and metastasis [129, 130, 133].

2.11.4 MMP-15 OR MT2-MMP

Six MT-MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, and MMP-25) comprise a small and distinct group among MMPs. They differ from soluble variants by their quality of remaining anchored to the cell membrane. MMP-15 is a ubiquitous enzyme which can activate MMP-2 pro-enzyme [126]. Physiological functions and the involvement of MMP-15 in pathology are not well described. In cancer cell line study of Abraham et al., MMP-15 was shown to have anti-apoptotic properties [134].

2.11.5 MMP-25 OR MT6-MMP

MMP-25 is a neutrophil-specific protease that was first detected in leukocytes [135]. MMP-25 has important functions in innate immunity due to regulating of the chemotaxis of neutrophils and monocytes [136]. Various cells can shed MMP-25 in exosomes, leading to paracrine transfer to other cells [126]. MMP-25 can degrade ECM proteins, including fibronectin, type IV collagen,

and proteoglycans [126]. A wide range of different cancers express MMP-25, although studies have not revealed yet the clinical relevance of the expression [126].

2.11.6 MMPS IN HEAD AND NECK CANCER

Table 3 shows immunohistochemical studies on MMPs and their associations with clinical factors in head and neck SCC. Studies on MMPs in SGC are infrequent. In acinic cell and mucoepidermoid carcinomas, MMP-7 immunoexpression and in epithelial-myoepithelial carcinomas MMP-9 immunoexpression have been related to better prognosis [122, 137]. On the contrary, abundant MMP-9 immunoexpression in ACC, mucoepidermoid carcinoma, acinic cell carcinoma, and salivary duct carcinoma has been related to poorer prognosis [138-141]. MMP-9 immunoexpression has been shown to be higher in ACC than in normal salivary gland tissue [140, 142].

Table 3. Immunohistochemical studies on matrix metalloproteinases and their clinical associations in oral and laryngeal squamous cell carcinomas.

	Tumor aggressiveness*	**Metastasis**	**Positivity in SCC surrounding inflammatory cells**	**Poor survival**	**No clinical associations**
Oral SCC	MMP-7 [123, 143, 144]	MMP-1, -2, -3, -7, -9, -14 [145] MMP-7 [123]	MMP-8 and -9 [144] MMP-8 [146]	MMP-9 [147]	MMP-25 [123]
Laryngeal SCC		MMP-15 [148] MMP-2, -7, -9 [149]		MMP-9 [147]	MMP-15 [150]

Abbreviations: SCC: Squamous cell carcinoma. MMP: Matrix metalloproteinase. * Tumor aggressiveness includes higher tumor grade and invasion depth.

2.12 ANTIZYME INHIBITORS (AZIN)

Enzymes called antizymes (AZ) and AZINs regulate ornithine decarboxylase (ODC), which is the rate-limiting enzyme of the biosynthesis of organic cations called polyamines [151, 152]. AZs down-regulate post-translationally ODC, whereas its upregulation is mediated by AZINs [153]. A mammalian cell uses polyamines for growth, proliferation, differentiation, and apoptosis. Consequently, their dysregulation is related to cancer, and

polyamine homeostasis has become an interesting target for cancer therapy [151, 153]. AZINs have not been previously studied in head and neck cancer or in SGC. New molecular therapies are warranted for SGC among ACC and functions of AZINs in ACC would be interesting to clarify.

2.12.1 AZIN1 AND AZIN2

AZIN1 was first described in 1980s [154]. AZIN1 has a stronger affinity to bind all three isoforms of AZs than ODC, leading to displacement of ODC from ODC-AZ heteromer and to reactivation of ODC [155]. Similarly, with ODC, AZIN1 is ubiquitous in all tissue types, and it is located in the nucleus and cytoplasm.

AZIN2 was first described at the beginning of the 2000s [156]. High amounts of AZIN2 are expressed in terminally differentiated cells such as neurons, mast cells, megakaryocytes in normal bone marrow, type-2 pneumocytes, adipocytes, and acinar cells of sweat glands [157]. AZIN2 is predominately expressed in the brain and testis, and it localizes in the endoplasmic reticulum-golgi intermediate compartment [156]. In the cell, AZIN2 regulates intracellular vesicle transport and the degranulation process of mast cells [152, 158].

2.12.2 AZINS IN CANCER

Dysregulation in polyamine homeostasis has been related to cancer progression [151, 153]. Overexpression of ODC enhances carcinogenesis, maintaining an invasive and angiogenic phenotype for a tumor cell [159]. Thus clearly, AZINs might contribute to carcinogenesis. AZIN1 elevates the activity of ODC, raising the number of intracellular polyamines that could trigger development of gastric, breast, hepatocellular and esophageal cancer [160]. In particular, disturbance in ribonucleic acid (RNA) editing of AZIN1 has been shown to participate in carcinogenesis in hepatocellular carcinoma [161]. AZIN2 is related to the poorer prognosis of colorectal cancer patients although specific molecular events are yet to be discovered [162].

3. AIMS OF THE STUDY

The study aimed to assess the factors determining the long-term outcome of minor salivary and mucous gland ACC patients. The presence of three polyomaviruses and 24 human papillomaviruses was assessed in the ACC tissue samples in order to study viral participation in ACC carcinogenesis. Knowing that finding new prognostic markers for ACC is warranted, the intention was to study the role of MMP-7, -8, -9, -15, and -25, and AZIN1 and -2 in minor salivary and mucous gland ACC.

Specific aims of the thesis study were as follows:

1. To evaluate the clinical and histological characteristics of minor salivary and mucous gland ACC during a 38-year period at the Helsinki University Hospital area in order to clarify their impact on patient survival (Study I).

2. To assess the presence of three polyomaviruses (JCPyV, BKPyV, and SV40) and 24 human papillomaviruses in minor salivary and mucous gland ACC (Study II).

3. To examine distinct biomarkers (MMP-7, -8, -9, -15, and -25) in the biology of ACC by immunohistochemistry and to relate the immunohistochemical results to clinical characteristics and outcome. In addition, to study associations of virus DNA-positive ACCs and MMPs (Study III).

4. To examine distinct biomarkers (AZIN1 and -2) in the biology of ACC by immunohistochemistry and to relate the immunohistochemical results to clinical characteristics and outcome (Study IV).

4. MATERIALS AND METHODS

All patients were diagnosed and treated at Helsinki University Hospital. Approximately 1.7 million people live in the referral area of Helsinki University Hospital.

4.1 STUDY I

A hospital record search found 86 MiSG ACC patients between 1974 and 2012. Sixty-eight patients were selected after ensuring adequate patient records and confirming and updating histological diagnoses according to the WHO classification (2005) by a head and neck pathologist (J.H.). Patient and tumor characteristics, treatment, and outcome were described. Tumor staging was performed according to the UICC TNM classification [52]. Statistics Finland provided causes and dates of death. All patients had a minimum follow-up time of three years or until death. OS, DSS, and DFS were defined from the last day of treatment to the last day of follow up or death (OS), to death due to disease (DSS), or to any sign of recurrent tumor (DFS).

4.2 STUDY II

Sixty-eight tumor samples from 53 MiSG ACC patients were available. The samples consisted of 48 primary tumors and in addition 20 recurrent tumors from 15 patients. Ten samples of normal salivary gland tissue from the same patients were used as controls. Tumor samples were studied to evaluate the presence of three polyomaviruses (JCPyV, BKPyV, and SV40) and 24 HPVs. Polyomaviruses were detected with quantitative polymerase chain reaction (qPCR) and positive samples were further studied for the presence of viral tumor T-ag by immunohistochemistry. Tumor samples were analyzed by Multiplex HPV Genotyping Kit for the presence of 24 mucosal alpha-HPV genotypes.

4.2.1 DNA EXTRACTION

Formalin-fixed and paraffin-embedded biopsy samples were cut into 5-μm-thick deparaffinized sections (1 cm^2 in total area) and DNA was extracted with the high salt method [163]. The sections were lysed in lysis buffer (10 mM Tris-HCl, 400 mM NaCl, and 2 mM EDTA, pH 8.2) with proteinase K (200

µg/ml) overnight at 37°C. Afterwards, proteins were precipitated with saturated NaCl and the DNA with ethanol.

4.2.2 QUANTITATIVE DETECTION OF JCPYV, BKPYV, AND SV40

Presence of JCPyV, BKPyV, and SV40 DNA in the samples was detected by qPCR (Roche, Light Cycler 96, Mannheim, Germany) targeting their oncogenic large T-ag. This method has been described earlier by McNees and coworkers and was performed here with a slight modification [164]. To ascertain a relative expression of the target genes, RNase P was used as a reference gene (TaqMan® Copy Number Reference Assay RNase, Applied Biosystems, Foster City, CA, USA).

The primers and probes were designed as described earlier [164] and produced by Life Technologies as outlined in Table 4. The probes for the target genes JCPyV, BKPyV, and SV40 were labeled with 6-carboxyfluorescein (FAM). VIC was used for labeling the probe for the reference gene RNase P. The qPCR reactions were performed in a 20 µl volume on a micro titer plate with conditions as follows: 900 nM of each primer and 100 nM of their analogous probe, 10 µl of TaqMan® Universal Mix II, and 300 ng template DNA. The manufacturer's recommendations were used to detect the reference gene TaqMan® RNase P (Applied Biosystems). The conditions for all qPCR reactions were the following: 2 min at 50°C, 10 min denaturation at 95°C, followed by 45 cycles of amplification with 95°C denaturation for 15s, and annealing/extension at 60°C for 60s. Amplification data measured as an increase in reporter fluorescence were collected in real time, and Roche, Light Cycler 96 software was utilized for data analysis.

The linear standard curves for JCPyV and BKPyV were obtained with a serial dilution of plasmids. For JCPyV amounts ranged from 1.2*10^2ng/µl to 1.2*10^-2 ng/µl and for BKPyV from 9.5*10^0 to 9.5*10^-3 ng/µl. The standards for SV40 detection were constructed with a serial dilution of COS1 cell line DNA containing one copy of SV40/cell. The number ranged from 5.0*10^4 to 5.0*10^0cells/µl. The standards for the reference gene RNase P were acquired with a serial dilution of human placenta DNA extractions (Sigma-Aldrich, Darmstadt, Germany) ranging from 5.09*10^2 to 5.09*10^-2. Cq values of less than 37 were considered to be a positive result, and the copy numbers were calculated as copies in 1 µg of human DNA.

Table 4. Primers and probes for JCPyV, BKPyV, and SV40.

Name	Sequence detection
JCPyV primer forward	TTC TTC ATG GCA AAA CAG GTC TT
JCPyV primer reverse	GAA TGG GAA TCC TGG TGG AA
BKPyV primer forward	CTT TCT TTT TTT TTT GGG TGG TGT T
BKPyV primer reverse	TTG CCA GTG ATG AAG AAG CAA
SV40 primer forward	GAT GGC ATT TCT TCT GAG CAA A

SV40 primer reverse	GAT GGC ATT TCT TCT GAG CAA A
JCPyV T-ag probe 6 FAM	CCA CTT CTC ATT AAA TG
BKPyV T-ag probe 6 FAM	AGT GTT GAG AAT CTG C
SV40 T-ag probe 5′-FAM	CAG GTT TTC CTC ATTAAA

Abbreviations: JCPyV: JC Polyomavirus. BKPyV: BK Polyomavirus. SV40: Simian vacuolating virus 40. T-ag: T antigen. FAM: 6-carboxyfluorescein. A: Adenine. C: Cytosine. G: Guanine. T: Thymine.

4.2.3 IMMUNOHISTOCHEMISTRY FOR JCPYV-POSITIVE TUMOR SAMPLES

Only, JCPyV-positive tumor samples were further analyzed with immunohistochemistry for the T-ag, as all the samples remained BKPyV and SV40 DNA negative. Sections (thickness 4 µm) of formalin-fixed and paraffin-embedded blocks were deparaffinized in xylene and rehydrated in a series of ethanol solutions. Thereafter, endogenous peroxidase activity was blocked by incubation of the slides with 3% hydrogen peroxide for 15 min. Epitope retrieval was performed using 1 mM citrate buffer, pH 6.0, and microwaved twice for 5 min.

The primary antibody was a mouse monoclonal anti-simian virus T-ag (1:75) (Anti-SV40 Antibody, clone Pab101, LifeSpan BioSciences Inc., Seattle, WA, USA) which cross-reacts with JCPyV T-ag. The tissue was incubated with the primary antibody overnight and detected with Dako REAL Detection System (Peroxidase/DAB+, Rabbit/Mouse, Dako, Glostrup, Denmark), followed by counterstaining with hematoxylin.

4.2.4 HPV DETECTION

Extracted DNA was amplified with primer sets 1 and 2 from the Multiplex HPV Genotyping Kit® (DiaMex GmbH, Heidelberg, Germany). Primer set 1 contained all HPV primers: nine biotinylated forward and three reverse primers for amplifying the HPV types under investigation. Primer set 2 (DNA quality control primers) contained primers for the amplification of a ß-globin gene fragment to verify the quantity and quality of human genomic sample DNA. A negative control contained no genomic DNA in order to confirm the absence of a contamination in the amplification reactions. Multiplex HPV Genotyping Kit® detected 24 low-risk (LR) and high-risk (HR) HPV genotypes as follows: LR-HPV6, -11, -42, -43, -44, and -70 and HR-HPV16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73, and -82. To analyze the labeled hybrids a Luminex LX-100 analyzer was used (Bio-Plex 200 System, Bio-Rad Laboratories, Hercules, CA, USA).

4.3 STUDY III

For the immunohistochemical study, tumor tissue samples were available from 52 patients. The samples consisted of 44 primary tumors and eight recurrent tumors. The immunoexpression profile of MMP-7, -8, -9, -15, and -25 was evaluated and immunoexpression of MMP-7, -9, -15, and -25 was associated with the clinicopathological factors and outcome.

4.3.1 METHODOLOGY FOR MMPS, EMA, AND CEA

Sections that were 4 μm thick cut from formalin-fixed and paraffin-embedded blocks were deparaffinized in xylene and rehydrated in graded ethanol and distilled water. Tissue slides were heated in a PreTreatment module (Agilent Dako, Santa Clara, CA, USA) in Tris-EDTA buffer, pH 9.0 (MMP-7, MMP-8, MMP-9) or in Tris–HCl buffer, pH 8.5 (MMP-15 and MMP-25) for 20 min at 98°C. Endogenous peroxidase activity was blocked by incubation of the slides with 0.3 % Dako REAL Peroxidase-Blocking Solution for 5 min and the primary antibody was diluted in Dako REAL Antibody Diluent. The primary antibodies were as follows: mouse monoclonal MMP-7 antibody (1:1000) (EMD Millipore Corporation, Temecula, CA, USA), rabbit polyclonal MMP-8 antibody (1:400) [165], rabbit polyclonal MMP-9 antibody (1:2000) (Calbiochem, Merck KGaA, Darsmstadt, Germany), mouse monoclonal MMP-15 antibody (1:250) (EMD Millipore Corporation, Temecula, CA, USA), and rabbit polyclonal MMP-25 antibody (1:300) (Abbexa, Cambridge, UK). Incubation of tissues with primary antibodies was for 1 h, albeit overnight for MMP-8, followed by detection with Dako REAL Detection System (Peroxidase/DAB+, Rab- bit/Mouse, Dako, Glostrup, Denmark). Slides were visualized by Dako REAL DAB + Chromogen or HRP Magenta Chromogen for 10 min and finally counterstained with hematoxylin (Mayer's Hematoxylin Dako, Glostrup, Denmark). Positive controls were oral mucosa and pancreas tissue for MMP-7, skin tissue for MMP-8, stomach and oral mucosa tissue for MMP-9, placenta and mammary gland tissue for MMP-15, and colon tissue for MMP-25.

Immunohistochemistry for EMA and CEA was performed in a routine laboratory with a Ventana Benchmark Ultra instrument (Roche, Tucson, AZ, USA). The slides were heated in a Ventana Cell Conditioning Solution (CC1) for 64 min for EMA and for 92 min for CEA. The primary antibody for EMA was clone E29, 790–4463 (ready-to-use), Ventana and for CEA clone II-7, M7072, (1:25), Dako. Incubation times were 40 and 60 min, respectively, followed by detection with a Ventana Ultraview DA, and finally counterstaining with hematoxylin.

4.3.2 IMMUNOSCORING

Blinded to the clinical data, two independent researchers (H.H. and J.H.) scored the slides. The proportion of positively stained cells was estimated and immunoscores were grouped as follows: 0 for negative or very mild (0–10%), 1 for mild (11–40%), 2 for moderate (41–70%), and 3 for strong (71–100%) positivity. This scoring method was modified from the previous publication of the Helsinki Head and Neck Research Group [123]. The location of immunoexpression at the cellular level (cytoplasm, nucleus, cell membrane, cell type) was analyzed. The immunoexpression of MMP-9 in tumor-surrounding inflammatory cells (PMN cells, lymphocytes, and plasma cells) and in luminal material of pseudocysts of ACC was additionally recorded. To validate the immunoexpression in normal tissue, normal salivary gland tissue present in the tumor slides was scored. EMA and CEA immunostainings were performed to ascertain the localization of MMP-9 immunopositivity in ACC tissues, particularly in relation to pseudocysts and true glands. EMA and CEA do not stain pseudocysts, although they are often positive in the true glands of ACC [166].

4.4 STUDY IV

Tumor tissue samples for AZIN1 immunohistochemical staining were available from 42 patients and comprised 35 primary and 7 recurrent tumors. For AZIN2, immunohistochemistry tumor samples were available from 45 patients cand comprised 37 primary and 8 recurrent tumors. Immunoexpression profile of AZIN1 and -2 was evaluated and compared with the clinicopathological factors and outcome.

4.4.1 METHODOLOGY FOR AZIN1 AND AZIN2

Four μm thick sections cut from formalin-fixed and paraffin-embedded blocks were deparaffinized in xylene and rehydrated in graded ethanol and distilled water. Tissue slides were heated in a PreTreatment module (Agilent Dako, Santa Clara, CA, USA) in antibody-specific buffer, pH 9 for 20 min at 98°C for antigen retrieval. Endogenous peroxidase activity was blocked by incubation of the slides with EnVision Flex peroxidase-blocking reagent for 15 min and the primary antibody was diluted in Dako REAL Antibody Diluent. The primary antibodies were as follows: rabbit polyclonal AZIN1 antibody (1:500) (Biorbyt Ltd, Cambridge, UK) and K3 antibody (1:600) [162]. The incubation times were overnight at +4°C and one hour at room temperature, respectively. This was followed by detection with the Dako REAL Detection System (Peroxidase/DAB+, Rabbit/Mouse, Dako, Glostrup,

Denmark), visualization with Dako REAL DAB+ Chromogen for 10 min, and counterstaining with hematoxylin (Mayer´s Hematoxylin Dako, Glostrup, Denmark). A positive control for AZIN1 was skin tissue and for AZIN2 gastric tissue. A slide without primary antibody was used as the negative control.

4.4.2 IMMUNOSCORING

Two independent researchers (H.H. and L.C.A.) performed scoring without knowledge of the clinical data. The location of immunoexpression on the different growth patterns (tubular, cribriform, and solid) was first analyzed. The proportion of positively stained tumor cells was estimated and immunoscores were grouped as follows: 0 for 0–10% (negative or very mild), 1 for 11–40% (mild), 2 for 41–70% (moderate), and 3 for 71–100% (strong). The scoring and grouping methods were modified from the previous publication of the Helsinki Head and Neck Research Group [123]. Normal salivary gland and apocrine gland tissue found on tumor slides was additionally immunoscored to validate immunoexpression in normal tissues.

4.5 STATISTICAL METHODS

IBM SPSS Statistics for Windows software was used for statistical analysis in Studies I, III, and IV (IBM Corp., Armonk, NY, USA, version 23.0 in Study I, version 25.0 in Studies III and IV). The Kaplan-Meier method was used for calculations of survival rates, and survival functions were compared with the log-rank test.

In Study I, Cox regression analysis was utilized in univariate associations of risk factors with OS, DSS, and DFS. The risk factors that were associated with OS, DSS, and DFS ($P < .05$ in univariate analysis) were used in multivariate Cox regression model. The stage was not included in the same multivariate model with T or N classes due to avoiding the multicollinearity problems. Results were reported by using hazard ratios with 95% confidence intervals.

In Studies III and IV, the Chi-squared test and Fisher´s exact test were used to evaluate the associations of MMP-7, -9, -15, and -25 and AZIN1 and -2 with clinicopathological factors. For statistical analysis, immunoscored tumor samples were grouped into different categories. In Study III, the categories were 0 (negative or very mild), 1 (mild), 2 (moderate), and 3 (strong), and in Study IV they were 0 (negative or very mild), 1 (mild), and 2-3 (moderate to strong). P-values less than .05 were considered statistically significant in all studies.

4.6 ETHICAL CONSIDERATIONS

The study concept was approved by the Institutional Research Ethics Board (Dnro 31/13/03/02/2010, 1 February 2010). For each study a research permit was obtained from the Department of Otorhinolaryngology at Helsinki University Hospital – Head and Neck Surgery. These retrospective studies consisted of reviewing hospital charts and histological specimens and did not have an effect on treatment of the patients included in the series. Therefore, patient consent was not required.

5. RESULTS

5.1 CHARACTERISTICS AND OUTCOME OF MISG ACC PATIENTS (STUDY I)

5.1.1 PATIENT AND TUMOR CHARACTERISTICS

The patient and tumor characteristics of 68 MiSG ACC patients are described in Table 5 based on the location of the primary tumor. The whole cohort consisted of 39 women (57%) and 29 men (43%); thus, a female/male ratio of 1.34. The median age was 58 years, ranging from 24 to 88 years. The majority of ACCs occurred in the oral cavity (41/68, 60%). Figure 7 shows the distribution of oral cavity ACCs. The most common location in the oral cavity was the palate (hard palate and the junction of the hard and soft palates) accounting for 49% of the intraoral ACCs occurred. Altogether 25% of tumors of the palate were stage I, 20% of stage II, 10% stage III, and 45% of stage IV tumors.

Table 5. Patient and tumor characteristics of 68 minor salivary gland ACC patients treated at Helsinki University Hospital between 1974 and 2012. Six tracheal tumors are excluded from the TNM and stage classification.

Location	Oral cavity	Oropharynx	Nasopharynx	Paranasal cavities	Larynx	Trachea	Esophagus	Ear
***N* (total 68)**	41	3	5	6	2	6	1	4
Age								
≤65 years	24	2	5	5	1	5	1	3
>65 years	17	1	-	1	1	1	-	1
Sex, women/men	19/22	3/0	4/1	2/4	2/0	6/0	0/1	3/1
T class								
T1	12	3	-	-	1		-	2
T2	12	-	-	-	-		-	-
T3	2	-	2	-	-		1	1
T4	12	-	2	6	1		-	1
N/A	3	-	1	-	-		-	-
N class								
N0	35	3	4	6	2		-	4
N1	1	-	-	-	-		-	-
N2	2	-	-	-	-		1	-
N/A	3	-	1	-	-		-	-
M class								
M0	36	3	4	6	2		1	4
M1	2	-	-	-	-		-	-
N/A	3	-	1	-	-		-	-
Stage								
I	10	3	-	-	1		-	2
II	12	-	-	-	-		-	-
III	3	-	2	-	-		1	1
IV	13	-	2	6	1		-	1
N/A	3	-	1	-	-		-	-
Size, (mm)	10-100	13-18	10-46	22-60	13-22	3-23	19	8-45
N/A	14	-	3	4	-	2	-	-
Resection margins								
Negative	18	1	1	-	1	1	-	1
Positive	13	1	2	3	-	2	1	3

N/A	10	1	2	3	1	3	-	-

Abbreviations: T: Tumor. N: Node. M: Metastasis. N/A: Not available.

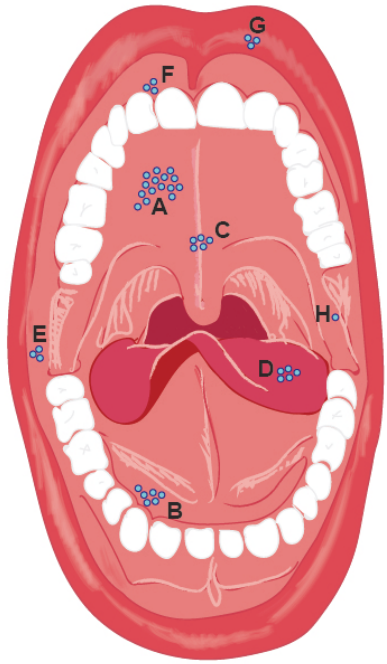

Figure 7. Distribution of 41 minor salivary gland adenoid cystic carcinomas of the oral cavity. A: Hard palate (n=15). B: Floor of mouth (n=6). C: Junction of hard and soft palate (n=5). D: Mobile tongue (n=5). E: Buccal mucosa (n=3). F: Gum (n=3). G: Upper lip (n=3). H: Mucous membrane of the oral cavity, not otherwise specified (n=1). Courtesy of Terhi Liuhto.

The primary symptoms were a lump (28%) and pain (18%). The duration of symptoms at the time of diagnosis had lasted from 1 to 240 months, with a median of 6 months. Three patients (4%) had a second primary malignancy at the time of diagnosis. The malignancies were prostate carcinoma, chromophobe renal cell carcinoma, and thyroid papillary microcarcinoma.

Prior to treatment, the most commonly used imaging methods were head and neck CT (58%) and MRI (46%). Other preoperative examinations were chest CT scan (31%), ultrasound (16%), fine needle aspiration (12%), and positron emission tomography CT (6%).

The median tumor size was 21 mm (range, 3-100 mm). The size was not available for 23 tumors (34%) in the clinical data or by histological re-evaluation. In the whole cohort, the most common growth pattern was cribriform (34%), followed by tubular (22%), combination of cribriform and tubular (19%), solid (9%), combination of cribriform and solid (7%), and combination of tubular and solid (2%). Growth pattern was not available in

five cases (7%). A combination growth pattern was considered unless a tumor sample comprised 80% of one growth pattern. Perineural or intraneural invasion, or both, was present in 39 tumors (57%). Information on neural invasion was not available for eight tumors (12%).

5.1.2 TREATMENT

The main treatment strategy was curative intent for 94% (64/68) of patients, 55% (35/64) of whom were treated with surgery as the sole treatment modality. Definite RT was given to one patient. Postoperative RT was given to 38% (24/64) of patients, and the median dose was 60 Gy (range, 32-70 Gy). Of patients, 8% (5/64) had chemotherapy during their oncological treatment.

Twenty-seven percent (17/64) of patients had neck dissection. Neck dissection was elective for 14 patients. Four patients had neck metastasis at the diagnosis. Three of these patients were treated with therapeutic neck dissection. Three patients with neck metastasis died due to ACC (range, 4-37 months), and one patient was alive at the end of the follow-up (39 months).

The treatment of ACC according to a tumor stage was relatively similar between different sites. Of patients with stage I and II tumors, 82% were treated with surgery alone. Only 22% of patients with advanced stage tumors (III-IV) were treated with surgery alone. Fifty-nine percent of patients with stage III and IV tumors received surgery combined with RT. Only 14% of patients treated with surgery and RT had stage I and II tumors. Patients who were offered oncological treatment had advanced ACCs in paranasal cavities and the nasopharynx, although one patient had stage I laryngeal ACC.

One patient treated with curative intent was lost to follow-up.

Altogether four patients (6%) received palliative treatment. Three of them had palliative surgery. One patient whose ACC was diagnosed in the 1970s did not receive any treatment.

5.1.3 OUTCOME FOR PATIENTS TREATED WITH CURATIVE INTENT

The 5-year OS and DSS rates were 70% and 79%, respectively. The 10-year OS and DSS rates were 42% and 52%, respectively.

Women and patients aged under 65 years had better OS (P= .018 and .005, respectively). T classes 2-4, stages II-IV, and N+ neck were associated with decreased OS (P= .005, .019, and .003, respectively) and DSS (P< .001, .001, and .001, respectively). In addition, T classes 2-4 and stages II-IV were associated with poorer DFS (P= .001 and .002, respectively). Comparison of stage I and stages II-IV tumors showed that patients with stage I tumor survived significantly better and none died due to ACC (Figure 8). Especially, treatment between low-grade (stages I and II) ACC patients did not differ considerably. In addition, neural invasion associated with poorer DFS (P= .016), but positive neural invasion was not an independent prognostic variable

in multivariate Cox regression analysis. Neural invasion was not associated with the rate of local or regional recurrent tumors.

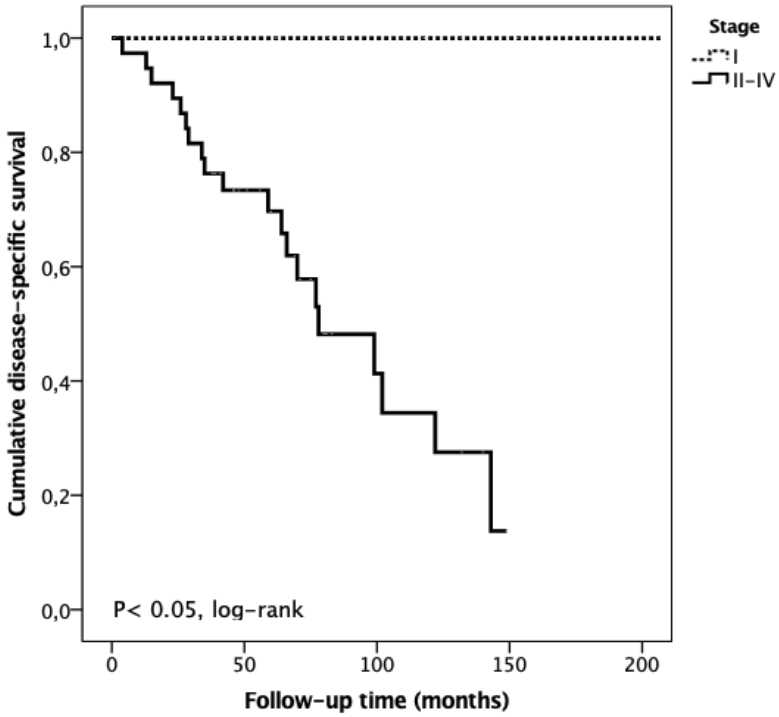

Figure 8. Kaplan-Meier plot showing a significant difference in the disease-specific survival of minor salivary gland adenoid cystic carcinoma patients with stage I compared with stage II-IV tumors.

5.1.4 RECURRENT TUMORS OF PATIENTS TREATED WITH CURATIVE INTENT

Overall, 53% (34/64) of patients treated with curative intent suffered from metastatic disease. Table 6 shows the distribution of recurrent tumors according to the primary tumor site for patients treated with curative intent. The majority (71%, 24/34) of recurrent tumors appeared within five years and 29% (10/34) later. The patients having recurrent ACC within five years had significantly poorer OS (P< .001) and DSS (P< .001) than patients with no metastasis. However, late metastasis compared with no metastasis had no effect on OS or DSS (P= .216 and .054, respectively). Local and regional recurrent tumors associated with decreased DSS (P= .042 and .020, respectively). Distant metastasis significantly associated with both poorer OS and DSS (P= .009 and .001, respectively). Most distant metastasis appeared within 10 years. Two of the distant metastases occurred more than 10 years later, one after 11 years and the other after 13 years.

Table 6. Characterization of the outcome of 64 patients with minor salivary gland adenoid cystic carcinoma treated with curative intent. One patient could have several recurrent tumors.

Tumor site	**Oral cavity**	**Oropharynx**	**Nasopharynx**	**Paranasal cavities**	**Larynx**	**Trachea**	**Esophagus**	**Ear**
Patients with metastasis/ All patients	**20/39**	**1/3**	**4/5**	**4/5**	**1/2**	**1/5**	**1/1**	**2/4**
Primary recurrence	7	0	2	4	0	1	0	2
Regional	4	0	0	0	0	1	0	0
Distant	14	1	4	1	1	1	1	0
Status								
NED	15	2	1	-	1	3	-	2
AWD	4	1	-	-	-	-	-	1
DOD	13	-	3	5	1	1	1	-
DOC	7	-	-	-	-	1	-	1
N/A	-	-	1	-	-	-	-	-

Abbreviations: NED: No evidence of disease. AWD: Alive with disease. DOD: Dead of disease. DOC: Dead of other cause. N/A: not available.

Stage I ACC patients

Three of 16 stage I patients treated with curative intent suffered from recurrent tumors: two local tumors (ACC in external ear canal) and one distant tumor (ACC in oropharynx). During the primary treatment 14 patients had surgery alone, one patient had surgery combined with RT (ACC in oropharynx), and one patient had chemoradiotherapy (ACC in larynx).

Neural invasion was detected in 30% of the tumors. Inadequate surgical margins, i.e. tumor-positive surgical margins were detected in five tumors (two in ear, two in oral cavity, and one in oropharynx). Seven tumors had adequate surgical margins, i.e. tumor-negative margins (six in oral cavity and one in oropharynx), and for four patients information was not available (two in oral cavity, one in larynx, and one in oropharynx). Positive surgical margins were detected in both patients with stage I ACC in the ear canal. They were not treated with postoperative RT. Unfortunately, these patients later suffered from local recurrent tumors. Despite additional RT to the patient with oropharyngeal ACC, a distant metastasis occurred in the lungs.

Altogether, despite a few recurrent tumors, patients with stage I ACC did not die due to ACC (range, 36-204 months). Four patients died due to causes other than ACC. Ten patients were alive with no evidence of ACC and two with disease at the end of the follow-up.

Stage II ACC patients

Eight of 12 patients with stage II ACC in the oral cavity suffered from recurrent tumors. Nine patients were treated with surgery alone and three with surgery combined with postoperative RT.

Neural invasion was detected in 67% of tumors. Surgical margin status was as follows: four tumor-positive and five tumor-negative surgical margins, and for three patients this information was not available.

During the follow up, five patients had died due to ACC (range 28-78 months) and one died due to another cause. Four patients were alive without recurrent tumors and two patients were alive with recurrent tumors.

Stage III ACC patients

Four of seven stage III patients had recurrent tumors. Surgery was offered to two patients with ACCs in the esophagus and oral cavity. Surgery combined with RT was offered to four patients with ACCs in the oral cavity (two), nasopharynx, and ear. One patient with nasopharyngeal ACC received only RT.

Neural invasion was detected in 57% of tumors. Surgical margin status was the following: three positive (ACCs in oral cavity, nasopharynx, and esophagus) and two negative surgical margins (ACCs in oral cavity and ear), and for two patients this information was not available (ACCs in oral cavity and nasopharynx).

All patients with recurrent ACC died due to the disease (range, 4-58 months), and the rest were alive with no evidence of ACC.

Stage IV ACC patients

Fourteen of 20 patients with stage IV ACC had recurrent tumors. Surgery alone was offered to four patients, surgery combined with RT to 12 patients, solely RT to one patient, and chemoradiotherapy to three patients.

Neural invasion was detected in 60% of tumors. Surgical margin status was the following: 10 positive (six in oral cavity, three in paranasal sinuses, and one in ear) and six negative surgical margins (four in oral cavity, one in larynx, and one in oropharynx), and for four patients this information was not available (two in paranasal sinuses, one in oral cavity, and one in nasopharynx).

Twelve patients died due to ACC (range, 13-143 months) and one due to another cause. Four patients were alive with no evidence of ACC and two patients with the disease. One patient was lost to follow-up.

5.2 JCPYV, BKPYV, SV40, AND HPVS IN MISG ACC (STUDY II)

The presence of JCPyV, BKPyV, SV40, and 24 mucosal alpha HPV DNA genotypes in ACC was assessed. Seven of the 68 samples (10%) contained JCPyV DNA positivity. The low viral load of JCPyV varied from 1 to 226 copies/μg DNA. Three samples showed slight nuclear immunopositivity for

large T-ag. Table 7 shows characteristics of patients with JCPyV DNA-positive tumors, and Figure 9 presents the locations of the tumors. Trachea, paranasal cavity, and oral cavity were the primary locations of JCPyV DNA-positive tumors. Furthermore, one JCPyV positive sample was derived from a lung metastasis of a tracheal tumor and one from the local recurrence of an oral cavity tumor.

None of the samples contained DNA of BKPyV, SV40, or any of the tested 24 HPV genotypes. Normal salivary gland control tissue samples were negative for JCPyV, BKPyV, and SV40.

Table 7. Characteristics of patients with JC polyomavirus DNA-positive adenoid cystic carcinomas.

Patient	Tumor location	Sex	Age	TNM	Treatment	Recurrence	Status
#1	Trachea	Woman	43		Surgery	Distant	DOC
#2	Trachea	Woman	54		Surgery		NED
#3	Trachea (lung metastasis)	Woman	47		Surgery	Distant, local, locoregional	DOD
#4	Paranasal sinuses	Man	60	$T_{4B}N_0M_0$	Oncological	Local	DOC
#5	Oral cavity, gum	Woman	80	$T_1N_0M_0$	Surgery		DOC
#6	Oral cavity, hard palate	Man	59	$T_{4B}N_0M_0$	Surgery and oncological		DOC
#7	Oral cavity, floor of mouth (local recurrence)	Woman	72	$T_{4A}N_0M1$	Surgery	Local	DOD

Abbreviations: T: Tumor. N: Node. M: Metastasis. DOC: Dead of other cause. NED: No evidence of disease. DOD: Dead of disease.

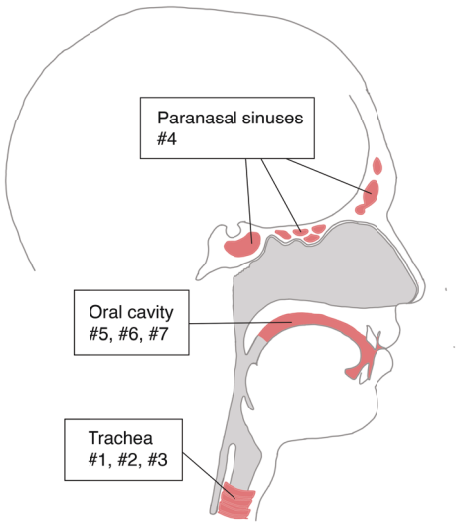

Figure 9. Locations of JCPyV DNA-positive adenoid cystic carcinomas. Courtesy of Ruusu Hulmi.

5.3 MMPS IN MISG ACC (STUDY III)

MMP-7, -8, -9, -15 and -25 immunoexpression in MiSG ACC was analyzed and examined in association with clinicopathological parameters and survival. Table 8 shows the distribution of the MMP immunoscoring. ACC did not immunoexpress MMP-8.

Table 8. Distribution of matrix metalloproteinase -7, -9, -15, and -25 immunoscoring in 52 minor salivary gland adenoid cystic carcinomas.

Immunoscore	0	1	2	3	N/A	Total	Luminal	Inf. cells	Normal SG
MMP-7	7 (13%)	15 (29%)	24 (46%)	4 (8%)	2 (4%)	52 (100%)	-	Neg.	Mild pos.
MMP-9	30 (57%)	17 (33%)	4 (8%)	0 (0%)	1 (2%)	52 (100%)	24 (46%)	29 (58%)	Mild pos.
MMP-15	32 (62%)	10 (19%)	5 (9%)	2 (4%)	3 (6%)	52 (100%)	-	Neg.	Mild pos.
MMP-25	4 (8%)	22 (42%)	17 (33%)	8 (15%)	1 (2%)	52 (100%)	-	Pos.	Neg.

Abbreviations: 0: Negative or very mild immunoexpression. 1: Mild immunoexpression. 2: Moderate immunoexpression. 3: Strong immunoexpression. N/A: Not available. Luminal: Immunoexpression in luminal material of many pseudocysts and a few true glands of ACC. Inf. cells: Immunoexpression in inflammatory cells. Normal SG: Immunoexpression in normal salivary gland. MMP: Matrix metalloproteinase.

5.3.1 ASSOCIATION BETWEEN MMPS AND CLINICOPATHOLOGICAL FACTORS

In Chi-squared test and Fisher´s exact test, high tumoral MMP-9 immunoexpression associated with advanced stage (P= .026) and with regional metastases (P= .035). MMP-9 positivity in luminal material of pseudocysts of ACC associated with better OS and DSS (P< .001) and with fewer local recurrent tumors (P= .031). MMP-9 positivity in inflammatory cells associated with immunopositivity in pseudocysts of ACC (P= .012) and with fewer local recurrent ACCs (P= .037). Abundant MMP-25 immunoexpression associated with better OS (P= .043).

In Kaplan-Meier survival analysis, abundant tumoral MMP-7 immunopositivity associated with better OS and DSS (P= .043 and .025, respectively). Abundant tumoral MMP-15 immunoexpression associated with poorer DSS (P= .041). High MMP-9 immunoexpression in pseudocysts of ACC associated with OS and DSS (P= .020 and .012, respectively).

The following clinicopathological factors were not significantly associated with any of the MMPs: gender, age, T, N, and M classification, neural invasion, and distant metastasis.

5.3.2 MMPS IN JCPYV DNA-POSITIVE SAMPLES

JCPyV DNA-positive and -negative samples were compared with immunoexpression of MMP-7, -9, -15, and -25 by using Fisher´s exact test. No statistically significant associations emerged with immunoexpression of MMP-7 and -9. Low MMP-15 and -25 immunoexpression associated with JCPyV DNA-positive samples (P= .004 and .041, respectively). However, there were only seven JCPyV DNA-positive samples.

5.4 AZINS IN MISG ACC (STUDY IV)

AZIN1 and -2 immunoexpression in MiSG ACC was analyzed and examined in association with clinicopathological parameters and survival.

5.4.1 IMMUNOEXPRESSION OF AZINS

Table 9 shows the distribution of AZIN1 and -2 immunoscoring. Immunohistochemistry of AZIN1 showed the strongest positivity in the cribriform growth pattern of ACC, followed by the tubular and solid patterns. Generally, AZIN1 immunoexpression was milder than AZIN2 immunoexpression. In normal structures, AZIN1 immunoexpression was present in apocrine glands, sweat glands, muscle cells, hair follicles, and germinal centers of lymphoid tissue.

AZIN2 immunohistochemical staining was strongest in the tubular growth pattern of ACC, followed by the cribriform and solid patterns. In normal structures, AZIN2 immunoexpression was present in mast cells, type 2 pneumocytes, neural structures, sweat glands, and minor salivary glands.

Table 9. Distribution of antizyme inhibitor 1 and 2 immunoscoring in minor salivary gland adenoid cystic carcinomas.

Immunoscore	0	1	2	3
AZIN1 (total 42)	13 (31%)	18 (43%)	9 (21%)	2 (5%)
AZIN2 (total 45)	2 (4%)	11 (25%)	24 (53%)	8 (18%)

Abbreviations: 0: Negative or very mild immunoexpression. 1: Mild immunoexpression. 2: Moderate immunoexpression. 3: Strong immunoexpression. AZIN: Antizyme inhibitor.

5.4.2 ASSOCIATION BETWEEN AZINS AND CLINICOPATHOLOGICAL FACTORS

In Chi-squared test and Fisher´s exact test, abundant immunoexpression of AZIN2 was associated with longer OS and DSS (P= .006 and P= .004, respectively).

In Kaplan-Meier survival analysis, abundant immunoexpression of AZIN2 associated with better DSS and DFS (P= .049).

The following clinicopathological factors were not associated with AZIN2: gender, age, T, N, and M classification, stage, neural invasion, and recurrent tumors. No significant associations were found within AZIN1 immunoexpression.

6. DISCUSSION

Among rare SGCs, ACC is the second most common histologic subtype. The long-term prognosis of ACC is poor. Thus, diagnosing MiSG ACC at an early stage, preferably at stage I, markedly benefits patient survival. Treatment of these tumors has been similar over the years, highlighting the importance of surgery and postoperative radiotherapy. However, studies on molecular pathogenesis and target-specific therapies are active.

In this thesis, the behavioral pattern of MiSG ACC was evaluated in a Finnish population (Study I). The presence of three polyomaviruses and 24 HPVs in MiSG ACC were assessed to study viral participation in the pathogenesis of ACC (Study II). Studies on MMPs in SGCs are sparse and this study aimed to investigate their prognostic role in the current cohort of MiSG ACCs (Study III). AZIN1 and -2 have not been previously evaluated in SGC or salivary gland tissue. Thus, an attempt was made to clarify the prognostic role of AZINs in ACC (Study IV).

6.1 PROGNOSTIC FACTORS FOR MISG ACC PATIENTS (STUDY I)

Based on Study I, the prognostic factors for MiSG ACC are gender, age, T and N classes, stage, neural invasion, and recurrent tumors.

Female gender predicting better OS was consistent with earlier studies on ACC [77, 167]. Aging has been previously related to poorer survival in ACC, similarly to other SGCs [29, 76, 77]. In the current study, age >65 years predicted poorer OS. Such predictive factors as gender and aging are not obviously only related to ACC because in general women have higher life expectancy and younger patients cope better with the side effects of cancer therapy.

Most of the MiSG ACCs occurred in the oral cavity (60%). The most common intraoral site was the palate (49%), as described earlier [45, 68-70, 142]. In the current study, 75% of palatal tumors were stages II-IV. The series of intraoral ACCs by Shum et al. demonstrated earlier that high numbers of palatal ACCs are diagnosed as advanced tumors [46]. The present and earlier results highlight the difficult and often late diagnosis of palatal ACCs. Importantly, a lump in the palate must always be further investigated to exclude the possibility of a salivary gland tumor.

In the current study, T classes 2-4 and stages II-IV associated with decreased OS, DSS, and DFS. Notably, patients with stage I ACC clearly had a better prognosis than patients with stage II ACC. Recurrent tumors affected only three of the 16 stage I patients, whereas eight of the 12 stage II patients had recurrent ACCs. In the case of positive surgical margins with stage I ACC,

it seemed that the postoperative RT was not necessary to avoid recurrent tumors if re-surgery was possible. Based on this patient cohort, stage II ACC appears to be a more unpredictable disease than stage I ACC. However, treatment between stage I and II ACC patients was quite similar. Therefore, adding treatment efforts to stage II patients and following them more closely could be beneficial for these patients. Earlier studies on MiSG ACC have concluded that early-stage ACC is a predictor of a better prognosis, although these studies have not emphasized a better prognosis for T1 and stage I tumors than for T2 and stage II tumors [44, 45, 68, 70].

With the complex anatomy of the ear, it seemed that postoperative RT would be feasible with positive surgical margins even with stage I tumors. In this study, two patients with stage I ACC in the external ear canal were not treated with postoperative RT, although the surgical margins were tumor-positive, leading to local recurrent tumors. With ACC in the external ear canal, local recurrencies have been shown to be associated with distant metastasis, occurring in 30% of the cases [168]. Zhang et al. concluded that postoperative RT was indicative for advanced ACC of the external ear canal [168]. In the present study, two patients with advanced ACC occurring in the external ear canal were treated with surgery and postoperative RT. Positive surgical margins were detected in one case. However, the treatment was successful and recurrent ACC was avoided.

In the present study, N+ neck at presentation predicted poorer OS and DSS. This finding correlates with earlier studies [68, 70]. In the present study, only four patients suffered from N+ neck and their advanced ACCs were located in the oral cavity and one in the esophagus. These patients were treated with neck dissection, but three of the four patients died due to ACC within three years. One patient was alive at the end of the follow up (39 months). Based on these findings, N+ neck is a predictor of a poor survival regardless of thorough treatment efforts.

Neurotropism is related to ACC, and in the current study neural invasion predicted poorer DFS. Similarly, earlier studies have shown that perineural invasion predicts poorer treatment outcome [169-171]. In this study, stages II-IV ACC presented more often with neural invasion than stage I ACC. ACC is a slow-growing SGC but with growing tumor size raises the possibility of detecting neural invasion.

In the present study, comparison of survival of patients with metastatic disease in relation to primary tumor site was not possible. The number of cases in a few sites was limited, and statistical power was insufficient for all sites. According to an earlier study, patients with oral cavity tumors have been shown to have superior survival to patients with tumors arising in MaSG, nasal cavity, larynx, and bronchus [77]. Moreover, poor survival has been shown with ACC occurring in sinonasal cavities [75, 76]. A patient might notice a tumor in the oral cavity easier and seek medical help. In addition, tumor-free surgical margins seem to be more difficult to achieve in the upper airways than in the oral cavity. The present study includes ACC patients from five decades,

reflecting the different treatment modalities over time. Nowadays sophisticated surgical approaches could be successful in the treatment of these high-risk sites.

The present study shows that regardless of the location, MiSG ACC metastasizes in 53% of cases. In the studies including both MiSG and MaSG ACCs, the proportion of metastasis has varied from 35% to 60% [8, 172]. In the present study, metastasis appeared more commonly within five years. The patients with early metastasis had decreased OS and DSS. Primary recurrencies and regional and distant metastases all had a negative effect on survival. Stages II-IV ACC produce more metastasis than stage I ACC.

Distant metastasis has been related to high-risk ACC locations such as the maxillary sinus and tongue [83] In this study, among curatively treated patients, distant metastasis appeared in 67% of patients who had metastasis. In the cohort of MaSG ACCs drawn from the same area and population as the present study, the rate of distant metastasis was 50% [173]. Similarly, Matsuba et al. have shown that 50% of MiSG and MaSG ACCs develop distant metastasis [28]. In this study, all distant metastasis appeared within 13 years. Close follow-up is feasible and the follow-up time should be at least 10 years.

6.2 VIRAL LOAD IN MISG ACC (STUDY II)

The presence of JCPyV, BKPyV, and SV40 and 24 alpha HPVs in ACC was studied and only JCPyV DNA (10%) was detected in the samples. These results indicate that the prevalence of JCPyV is infrequent in MiSG ACC. However, the viral presence could be one step towards malignancy. Given that, JCPyV could contribute to the pathogenesis of a small proportion of ACCs.

The seroprevalences of BKPyV and JCPyV are common among the adult population, being 82-99% and 39-81%, respectively [89], but also among children [90]. As the infection with these two viruses classified as grade 2B carcinogens is so common, it is tempting to speculate their possible role also in several carcinomas among SGCs. BKPyV is shed in saliva and viral replication is shown to occur *in vitro* in salivary gland cells [174]. In the head and neck area, tonsil tissue has been proposed to serve as a reservoir for BKPyV and JCPyV, which have been detected to some extent in the oropharynx [99, 100]. JCPyV, BKPyV, and SV40 have also been detected in salivary gland tumors [108], [112] but none of these tumors were ACCs. However, the results are still contradictory. Ramqvist et al. did not detect any BKPyV or JCPyV in their series of different SGCs, but MCPyV was present in three SGCs, including one ACC [175]. They studied 10 HPyV types from parotid gland SGCs, with SGC subtypes varying from one to 19 [175]. In the present study, the total number of ACCs was representative (n=68) with 10% of JCPyV positive tumors. Previously, inoculation of a mouse polyomavirus to MaSG of a mouse resulted in a benign tumor formation, resembling a pleomorphic adenoma [111]. Sandros

et al. inoculated polyoma virus into newborn mice, which developed adenocarcinomas in less than three months. Typical for these tumors was viral integration and karyotypic instability [176].

Despite the evidence from animal models of polyomavirus inoculation leading to tumor formation, the role for JCPyV in human cancers and tumor formation is unclear and remains a subject of debate [177]. After primary infection, JCPyV latency is described in the kidney and urothelial tissue, and JCPyV DNA is detected in normal colonic mucosa [178]. Furthermore, JCPyV has been detected in urothelial carcinoma [178], in brain tumors [179], in colorectal neoplasia of liver transplant patients [180], and in cancers of the gastrointestinal tract [181], while others report no correlation [177].

The incidence of SGC is increasing [182-184], but the reason for this trend remains obscure. Possible explanations could be improvements in diagnosing different subtypes of SGC, and yet unknown risk factors and their role in activating or establishing the latency of oncoviruses. Approximately 8.3% of human cancers are caused by oncoviruses [92]. Studies on viral etiology of salivary gland tumors are often focused on salivary gland tumors as such but not on a specific malignant tumor type. As known, the molecular landscape of SGC subtypes is different, indicating that their pathogenesis differs from each other. Clarifying the prevalence, viral physical state, and viral transcription pattern of all potential oncoviruses according to the SGC entity would cast more light on this complex issue. Pathogenesis of SGC could perhaps be different between MiSGs and MaSGs due to their different anatomical locations and their physical features (e.g. secretion).

In general, the number of virus-related head and neck cancers has increased especially due to HPV infection. In the present study, HPV DNA was not detected in the ACC samples. Compared with previous studies on SGC, the current series with ACC as the only tumor entity is representative. There are only few studies on the role of HPVs in SGCs [30-32, 116, 185-189]. Haeggblom et al. did not detect HPVs in salivary gland tumors including 13 ACCs [31], confirming the findings from Skálová et al. [30] and Bishop et al. [116]. Qian et al. detected HPVs in 42% of ACCs [186]. HPV DNA-positive tumors and negative resection margins predicted better recurrence-free survival. The association with HR-HPVs was slightly stronger in MaSG ACCs (44%) than in MiSG ACCs (38%) [186]. To conclude, the studies on the association between HPV and SGC are scarce, and the results are conflicting [30-32, 116, 185-189]. The present data do not support a specific role of HPV in the pathogenesis of ACC.

6.3 MMPS IN MISG ACC (STUDY III)

In this study, MMP-9 immunoexpression associated with poorer prognosis such as advanced stage and regional metastasis. Similar findings with adverse effects of MMP-9 immunoexpression have been shown previously. High

MMP-9 immunoexpression in ACC has been established to be related to poorer prognosis such as advanced stage, solid growth pattern, perineural invasion, recurrences, and shorter survival time [140]. Souza Freitas et al. have shown abundant MMP-9 immunoexpression in ACC compared with a normal salivary gland [142]. Interestingly, in this study, MMP-9 immunoexpression in the luminal material of pseudocysts of ACC and in inflammatory cells close to the tumor associated with better prognosis. MMP-9 immunoexpression in pseudocysts associated with better survival and fewer local recurrent tumors, while immunoexpression in inflammatory cells associated with fewer local recurrent tumors and positivity in pseudocysts. Possibly within these tumors, MMP-9 was not active when located in the luminal material of pseudocysts or in inflammatory cells. Pujada et al. have suggested that in colitis-associated colorectal cancer MMP-9 might act as a tumor suppressor [190], which is contrary to the current knowledge of the role of MMP-9 in tumor invasion and metastasis [130].

In the present study, abundant MMP-7 immunoexpression associated with longer OS and DSS. This was consistent with the results of Luukkaa et al. of an association between MMP-7 immunoexpression and better prognosis of SGC [122].

This study was the first to demonstrate an MMP-15 and MMP-25 immunoexpression pattern in SGC and in normal salivary gland tissue. Abundant MMP-15 immunoexpression associated with decreased survival time. Previously, abundant MMP-15 immunoexpression in laryngeal SCC has been related to metastatic behavior [148]. In the present study, high immunoexpression of MMP-25 in ACC associated with better survival. Previous studies examining the relationship between MMP-25 and head neck cancer are limited [123]. ACC cells did not immunoexpress MMP-8.

Only a few studies have been examined the functions of MMPs in the carcinogenesis and microenvironment of SGC [122, 138-142, 191-193]. Specific behavior of MMPs in SGCs among ACC is not fully understood, but MMPs could function especially during metastasis. MMPs can process ECM and non-matrix bioactive molecules (cytokines, hormones, defensins, immune mediators, other proteases, adhesion molecules, growth factors, and cell surface receptors). Due to this ability, MMPs are important in a cancer cell. Metastasis is a multistep process that includes decreased cell adhesion, increased motility and invasion, proteolysis, and resistance to apoptosis. Especially, interactions between MMPs and claudins (cell membrane protein family) might play an important role in invasive behavior of cancer cells and metastasis. [194]

To study the relations between virus DNA-positive samples and immunoexpression of MMPs, this study associated MMP immunoexpression with JCPyV DNA-positive and -negative samples. With JCPyV DNA-positive samples, MMP-15 and MMP-25 immunoexpressions were significantly weaker. Previously, Liu et al. have been interested in the functions of MMPs in MCPyV infection. They studied whether MMP-mediated degrading of ECM

could stimulate MCPyV infection, leading to tumorigenesis of MCC [195]. They showed that induction of MMP genes by the WNT/Beta-catenin signaling pathway and other growth factors stimulate MCPyV infection. The transcription of several MMP genes (*MMP-1*, *MMP-3*, *MMP-7*, *MMP-9*, *MMP-10*, *MMP-11*, and *MMP-13*) was increased in dermal cells. However, no increase in mRNA levels of *MMP-15* and *MMP-25* genes was found. [195] In the current study, within JCPyV DNA-positive samples, MMP-15 and MMP-25 immunoexpressions were weaker than in virus-negative samples. MMP-7 and MMP-9 immunoexpressions were not associated with the virus DNA-positive samples. Previously, an association has been shown between MMP-9 and JCPyV reactivation during the treatment of multiple sclerosis patients with humanized monoclonal antibody against the cell adhesion molecule α4-integrin (natalizumab) [196]. MMP-9 plasma level was shown to increase at the time of JCPyV reactivation [196].

This study showed that ACC tissue immunoexpressed MMPs. MMPs are known to activate each other (e.g. MMP-7 activates MMP-8 and -9), and they stimulate cascades that may promote tissue modulation and metastatic potential of cancer cells. In the future, additional studies on the role of MMPs in SGC are warranted.

6.4 AZINS IN MISG ACC (STUDY IV)

In this study, high AZIN2 immunoexpression associated with longer OS and DSS. AZIN1 immunoexpression did not have any statistically significant associations with the studied clinicopathological factors and survival. Recently, AZIN2 has been related to poorer prognosis of colorectal adenocarcinoma [197], which was the reason for conducting this study on AZINs in salivary gland adenocarcinoma. However, specific molecular events have not yet been revealed behind AZIN2. Furthermore, recently, Shigeyasu et al. have shown that epigenetic modification (RNA editing) of AZIN1 in colorectal cancer seems to drive the metastatic process and could be used as a prognostic factor [198].

In the current study, AZIN1 and AZIN2 were seen in tubular and cribriform growth patterns with secretory functions rather than in the solid pattern. This finding is similar to that of Kaprio et al. [197], who described abundant AZIN2 immunoexpression in mucinous colorectal cancer tissue with secreting functions. Solid ACC is poorly differentiated, and it might have lost some of the normal cell functions, e.g. the vesicle transportation system. In tubular and cribriform patterns, ductal structures formed by luminal (epithelial) cells are numerous compared with the solid pattern, which is mainly composed of basal/myoepithelial cells with a few ductal structures.

This study was the first to show AZIN1 and -2 in salivary gland carcinoma and in normal salivary gland tissue. MiSG structures representing normal

salivary gland tissue in the immediate vicinity of ACC lacked the immunoexpression of AZIN1. However, AZIN2 immunoexpression was present in ductal structures of MiSG. Previously, abundant AZIN2 expression has been shown in terminally differentiated cells, namely neurons, adipocytes, acinar cells of sweat glands, and megakaryocytes in bone marrow [199]. Interestingly, AZIN2 has been shown to regulate vesicle transportation in a cell and mast cell degranulation process [200, 201].

AZINs have an important role in cellular functions, contributing to polyamine synthesis. AZIN1 has been studied more than AZIN2. In pathological circumstances, AZIN1 has been related to formation of gastric, breast, hepatocellular and esophageal cancers [160]. Specific molecular events of AZINs in normal and cancer cells warrant clarification. Both AZINs have been proposed for use as therapeutic agents against cancer [153, 198].

6.5 LIMITATIONS AND STRENGTHS OF THE THESIS

Study I included patients from one tertiary care academic center, and the number of patients was large compared with previous studies on MiSG ACC. The study material was partly old and collecting data on all cases was not possible. For example, details of vascular invasion and surgical margins were not always reported. Statistical analysis based on these parameters could not be conducted. In the cohort, MiSG ACCs from eight different subsites were studied. In a few locations, such as paranasal sinuses, the number of cases was limited in order to statistically evaluate separately anatomical subsites. Within the long period of the retrospective study, the diagnostic assessment and treatment modalities have evolved. One patient was lost to follow-up due to moving to another European country. However, the follow-up time for all the patients was at least three years.

In Study II, viral load of MiSG ACC samples was studied. The total sample size of this one tumor entity was large relative to previous SGC studies on oncoviruses. qPCR method is reliable for detecting viral load in tissue samples. JCPyV DNA was detectable, but levels were quite low. Detection of JCPyV DNA by immunohistochemistry proved to be challenging due to small viral load.

In Studies III and IV, immunoexpressions of biomarkers (MMP-7, -8, -9, -15, and -25 and AZIN1 and -2) were related to clinicopathological parameters and survival. Unfortunately, tissue samples from all cases were not available, likely because some of the oldest tissue samples had already been exhausted. The small sample size in the studies of rare diseases, such as ACC, is a common limitation. Other limitation is that determining the proportion of positive staining is subjective. To avoid bias in evaluating the immunohistochemical

staining, two researchers performed the scoring separately, afterwards comparing their results.

7. CONCLUSIONS

1. Stage II MiSG ACC patients suffered from more unpredictable and aggressive disease than stage I patients. Treatment strategy of stage II ACC patients should be customized, as these patients have advanced disease. Of palatal ACCs, 75% were stages II-IV, which demonstrates the difficulty of the early diagnosis. Clinicians should always consider a lump in the palate as a possible salivary gland tumor and further investigate the area.

Older age, advanced T class and stage, N+ neck at diagnosis, and locoregional and distant metastases had negative associations with survival. Of the patients, 53% developed metastases during the follow-up. Distant metastases appeared within ten years, except for two patients with manifestation within 13 years. Thus, prolonged follow-up, (at least ten years), is recommended.

2. JCPyV DNA was detected from MiSG ACC samples in the upper and lower airways. However, BKPyV, SV40, and HPVs were not found. JCPyV could be a part of tumorigenesis of ACC in a small proportion of ACC patients. While the presence of low copy numbers of JCPyV DNA in itself does not establish causation, one cannot exclude the viral role in early malignant transformation according to the hit and run model of carcinogenesis.

3. MMP-7, -9, -15, -and 25 were immunoexpressed in MiSG ACC. Inflammatory cells immunoexpressed MMP-8, -9, and -25. High tumoral MMP-9 and MMP-15 immunoexpression associated with poorer prognosis. In contrast, high tumoral MMP-7 and MMP-25, and high MMP-9 in the luminal structures of pseudocysts of ACC and in inflammatory cells surrounding the tumor, associated with better prognosis. MMPs are known to function in different activating cascades and in immunomodulation. Thus MMP-7, -8, and -9 can take part in tissue modulation and in the metastatic process. MMP-15 and -25 are related to prognosis.

4. This study was the first to show AZIN1 and -2 immunoexpression patterns in ACC. Both AZINs were markedly present in well-differentiated growth patterns, namely tubular and cribriform patterns compared with the solid growth pattern. Abundant AZIN2 immunoexpression associated with longer OS and DSS. With the knowledge that AZIN2 contributes to vesicle transportation, it may be speculated that AZIN2 participates in vesicle transportation in well-differentiated ACC cells.

ACKNOWLEDGMENTS

This work was conducted between years 2014 and 2021 at the Department of Pathology, Helsinki University Hospital in collaboration with the Department of the Otorhinolaryngology – Head and Neck Surgery and the Department of Oral Pathology and Radiology, University of Turku.

This work was financially supported by grants by Helsinki University Hospital Research Fund, Cancer Foundation Finland, The Finnish Dental Society Apollonia, and Suomen Naishammaslääkärit Ry. I sincerely acknowledge them all.

I am grateful for many persons for all the support, good advices and collaboration during this thesis project. First of all, I want to express my deepest gratitude to my supervisors Professor Jaana Hagström, Professor Antti Mäkitie, and Docent Leif Bäck. Jaana, you started recruiting me to start research in the beginning of dental school. I am so grateful for always knowing better, even back then. It has been great to have such a supportive, helpful, firm, daring, and warm person to guide me through the process. Antti, thank you for sharing many wise words, time, support, and patience. Lefa, thank you for your support, clinical expertise and especially skills in statistics. Without you, all the SPSS work I have ever made could not have been possible.

I want to thank my official reviewers, Docents Heikki Teppo and Tero Soukka for their constructive comments, responsiveness in a short schedule, and encouraging attitude. I warmly thank the excellent thesis committee members, Docents Jaana Rautava and Kimmo Suomalainen. Thank you for objectiveness and reviewing my thesis project with expertise.

I sincerely thank all my coauthors. PhD, MD Karoliina Hirvonen, thank you for warmly welcoming me to this minor salivary gland ACC study, which started as part of your thesis project. Thank you for all the educative and also fun talks and brainstorming. Professor Stina Syrjänen, thank you for sharing your expertise, enthusiasm, and educative approach during my thesis study and also in the specialist training. Professor Ilmo Leivo, Professor Timo Sorsa, and Professor Leif C. Andersson, thank you for sharing your time and enormous expertise. Professor Caj Haglund, Docent Kauko Saarilahti, and PhD, DDS Satu Apajalahti, thank you for your kind collaboration.

I extend my gratitude to Pia Saarinen for the technical assistance with immunostainings and Tatjana Peskova for the PCR analyses. Ruusu Hulmi and Terhi Liuhto, thank you for creating beautiful images for my thesis.

My dear colleagues and friends, it really warms my heart to know I have such excellent colleagues and good friends. I would especially want to thank oral pathologists Jetta Kelppe, Jaana Willberg, Tuula Salo, Aleksi Rytkönen, Maria Siponen, Mirja Puranen, Kristiina Heikinheimo, Saara Kantola, Heidi Harinen, and the ones mentioned earlier. I feel privileged to be a part of this network of experts.

Finally, I want to thank my parents, Sirpa and Harri, and my sister Heidi for being always there for me giving their unconditional love and support. My

mother-in law Anja, I am grateful for our good relations and your wonderful help whenever needed, especially with our children. My beloved JP, thank you for being the greatest husband I could ever hope for and a wonderful father to our children Mauno and Monna.

REFERENCES

1. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, editors. WHO Classification of Head and Neck Tumours. 4th ed. Lyon, France: IARC Press; 2017.

2. Luukkaa H, Klemi P, Leivo I, et al. Salivary gland cancer in Finland 1991--96: an evaluation of 237 cases. Acta Otolaryngol. 2005;125:207-214.

3. Bjørndal K, Krogdahl A, Therkildsen MH, et al. Salivary gland carcinoma in Denmark 1990-2005: a national study of incidence, site and histology. Results of the Danish Head and Neck Cancer Group (DAHANCA). Oral Oncol. 2011;47:677-682.

4. Iqbal H, Bhatti AB, Hussain R, Jamshed A. Survival outcome of malignant minor salivary tumors in Pakistani population. South Asian J Cancer. 2014;3:163-165.

5. Parikh AS, Khawaja A, Puram SV, et al. Outcomes and prognostic factors in parotid gland malignancies: A 10-year single center experience. Laryngoscope Investig Otolaryngol. 2019;4:632-639.

6. da Silva LP, Serpa MS, Viveiros SK, et al. Salivary gland tumors in a Brazilian population: A 20-year retrospective and multicentric study of 2292 cases. J Craniomaxillofac Surg. 2018;46:2227-2233.

7. Coca-Pelaz A, Rodrigo JP, Bradley PJ, et al. Adenoid cystic carcinoma of the head and neck--An update. Oral Oncol. 2015;51:652-661.

8. de Morais EF, da Silva LP, Moreira DGL, et al. Prognostic factors and survival in adenoid cystic carcinoma of the head and neck: A retrospective clinical and histopathological analysis of patients seen at a cancer center. Head Neck Pathol. 2021;15:416-424.

9. Geiger JL, Ismaila N, Beadle B, et al. Management of salivary gland malignancy: ASCO guideline. J Clin Oncol. 2021;39:1909-1941.

10. Hay AJ, Migliacci J, Karassawa Zanoni D, McGill M, Patel S, Ganly I. Minor salivary gland tumors of the head and neck-Memorial Sloan Kettering experience: Incidence and outcomes by site and histological type. Cancer. 2019;125:3354-3366.

11. He JF, Lin Y, Wang B, Zhu WY, Wei D, Zhu HY. Analysis of the clinicopathological characteristics and prognosis of adenoid cystic carcinoma of the intraoral minor salivary glands: a retrospective study of 40 cases. Int J Oral Maxillofac Surg. 2021;50:444-450.

12. Sakata R, Preston DL, Brenner AV, et al. Radiation-Related Risk of Cancers of the Upper Digestive Tract among Japanese Atomic Bomb Survivors. Radiat Res. 2019;192:331-344.

13. Horn-Ross PL, Ljung BM, Morrow M. Environmental factors and the risk of salivary gland cancer. Epidemiology. 1997;8:414-419.

14. Radoï L, Barul C, Menvielle G, et al. Risk factors for salivary gland cancers in France: Results from a case-control study, the ICARE study. Oral Oncol. 2018;80:56-63.

15. Falchook AD, Zevallos JP, Chera BS. Increased risk of salivary gland cancer among women with a previous cancer diagnosis. Head Neck. 2016;38 Suppl 1:E446-51.

16. Persson M, Andrén Y, Mark J, Horlings HM, Persson F, Stenman G. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proc Natl Acad Sci U S A. 2009;106:18740-18744.

17. de Almeida-Pinto YD, Costa SFDS, de Andrade BAB, et al. t(6;9)(MYB-NFIB) in head and neck adenoid cystic carcinoma: A systematic review with meta-analysis. Oral Dis. 2019;25:1277-1282.

18. Skálová A, Stenman G, Simpson RHW, et al. The Role of Molecular Testing in the Differential Diagnosis of Salivary Gland Carcinomas. Am J Surg Pathol. 2018;42:e11-e27.

19. Valstar MH, de Bakker BS, Steenbakkers RJHM, et al. The tubarial salivary glands: A potential new organ at risk for radiotherapy. Radiother Oncol. 2021;154:292-298.

20. Vander Poorten V, Hunt J, Bradley PJ, et al. Recent trends in the management of minor salivary gland carcinoma. Head Neck. 2014;36:444-455.

21. Gnepp DR. Diagnostic Surgical Pathology of the Head and Neck. 2nd ed. Philadelphia, PA, USA: Saunders Elsevier; 2009.

22. Stoeckelhuber M, Matthias C, Andratschke M, et al. Human ceruminous gland: ultrastructure and histochemical analysis of antimicrobial and cytoskeletal components. Anat Rec A Discov Mol Cell Evol Biol. 2006;288:877-884.

23. de Paula F, Teshima THN, Hsieh R, Souza MM, Nico MMS, Lourenco SV. Overview of human salivary glands: Highlights of morphology and developing processes. Anat Rec (Hoboken). 2017;300:1180-1188.

24. Barnes L, Eveson WJ, Reichart P, Sidransky D, editors. Pathology and Genetics of Head and Neck Tumours. Lyon, France: IARCPress; 2005.

25. Ettl T, Schwarz-Furlan S, Gosau M, Reichert TE. Salivary gland carcinomas. Oral Maxillofac Surg. 2012;16:267-283.

26. Goel AN, Badran KW, Braun APG, Garrett AM, Long JL. Minor salivary gland carcinoma of the oropharynx: A population-based analysis of 1426 patients. Otolaryngol Head Neck Surg. 2018;158:287-294.

27. Spiro RH. Salivary neoplasms: overview of a 35-year experience with 2,807 patients. Head Neck Surg. 1986;8:177-184.

28. Matsuba HM, Thawley SE, Simpson JR, Levine LA, Mauney M. Adenoid cystic carcinoma of major and minor salivary gland origin. Laryngoscope. 1984;94:1316-1318.

29. Israel Y, Rachmiel A, Gourevich K, Nagler R. Salivary malignancies-medical, demographic and diagnostic analysis. J Craniomaxillofac Surg. 2019;47:500-504.

30. Skálová A, Kašpírková J, Andrle P, Hostička L, Vaneček T. Human papillomaviruses are not involved in the etiopathogenesis of salivary gland tumors. Cesk Patol. 2013;49:72-75.

31. Haeggblom L, Ursu RG, Mirzaie L, et al. No evidence for human papillomavirus having a causal role in salivary gland tumors. Diagn Pathol. 2018 Jul 18;13(1):44. doi: 10.1186/s13000-018-0721-0

32. Hühns M, Simm G, Erbersdobler A, Zimpfer A. HPV infection, but not EBV or HHV-8 infection, is associated with salivary gland tumours. Biomed Res Int. 2015;2015:829349.

33. Xu B, Drill E, Ho A, et al. Predictors of outcome in adenoid cystic carcinoma of salivary glands: A clinicopathologic ttudy with correlation between MYB fusion and protein expression. Am J Surg Pathol. 2017;41:1422-1432.

34. Mitani Y, Li J, Rao PH, et al. Comprehensive analysis of the MYB-NFIB gene fusion in salivary adenoid cystic carcinoma: Incidence, variability, and clinicopathologic significance. Clin Cancer Res. 2010;16:4722-4731.

35. West RB, Kong C, Clarke N, et al. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. Am J Surg Pathol. 2011;35:92-99.

36. Thierauf J, Ramamurthy N, Jo VY, et al. Clinically integrated molecular diagnostics in adenoid cystic carcinoma. Oncologist. 2019;24:1356-1367.

37. Simpson RH, Skálová A, Di Palma S, Leivo I. Recent advances in the diagnostic pathology of salivary carcinomas. Virchows Arch. 2014;465:371-384.

38. Bell D, Hanna EY, Miele L, Roberts D, Weber RS, El-Naggar AK. Expression and significance of notch signaling pathway in salivary adenoid cystic carcinoma. Ann Diagn Pathol. 2014;18:10-13.

39. Stephens PJ, Davies HR, Mitani Y, et al. Whole exome sequencing of adenoid cystic carcinoma. J Clin Invest. 2013;123:2965-2968.

40. Chen W, Cao G, Yuan X, et al. Notch-1 knockdown suppresses proliferation, migration and metastasis of salivary adenoid cystic carcinoma cells. J Transl Med. 2015 May 20;13:167.

41. Ferrarotto R, Mitani Y, Diao L, et al. Activating NOTCH1 mutations define a distinct subgroup of patients with adenoid cystic carcinoma who have poor prognosis, propensity to bone and liver metastasis, and potential responsiveness to Notch1 inhibitors. J Clin Oncol. 2017;35:352-360.

42. Holst VA, Marshall CE, Moskaluk CA, Frierson HF,Jr. KIT protein expression and analysis of c-kit gene mutation in adenoid cystic carcinoma. Mod Pathol. 1999;12:956-960.

43. Monteiro LS, Bento MJ, Palmeira C, Lopes C. Epidermal growth factor receptor immunoexpression evaluation in malignant salivary gland tumours. J Oral Pathol Med. 2009;38:508-513.

44. Michel G, Joubert M, Delemazure AS, Espitalier F, Durand N, Malard O. Adenoid cystic carcinoma of the paranasal sinuses: retrospective series

and review of the literature. Eur Ann Otorhinolaryngol Head Neck Dis. 2013;130:257-262.

45. DeAngelis AF, Tsui A, Wiesenfeld D, Chandu A. Outcomes of patients with adenoid cystic carcinoma of the minor salivary glands. Int J Oral Maxillofac Surg. 2011;40:710-714.

46. Shum JW, Chatzistefanou I, Qaisi M, Lubek JE, Ord RA. Adenoid cystic carcinoma of the minor salivary glands: a retrospective series of 29 cases and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;121:210-214.

47. Dardick I. Color Atlas/Text of Salivary Gland Tumor Pathology. 1th ed. New York, USA: IGAKU-SHOIN Medical Publishers, Inc; 1996.

48. Raman S, Sherlin HJ. Utility of smooth muscle actin and CD117 as reliable markers in the diagnosis of salivary gland neoplasms. J Oral Maxillofac Pathol. 2019;23:218-223.

49. Ettl T, Schwarz S, Kleinsasser N, Hartmann A, Reichert TE, Driemel O. Overexpression of EGFR and absence of C-KIT expression correlate with poor prognosis in salivary gland carcinomas. Histopathology. 2008;53:567-577.

50. Chen JC, Gnepp DR, Bedrossian CW. Adenoid cystic carcinoma of the salivary glands: an immunohistochemical analysis. Oral Surg Oral Med Oral Pathol. 1988;65:316-326.

51. Adelstein DJ, Koyfman SA, El-Naggar AK, Hanna EY. Biology and management of salivary gland cancers. Semin Radiat Oncol. 2012;22:245-253.

52. Brierley J, Gospodarowicz M, Wittekind C, editors. TNM Classification of Malignant Tumours. Wiley Blackwell; 2016. UICC, Eight Edition.

53. Kumar NS, Iype EM, Thomas S, Sankar UV. Adenoid cystic carcinoma of the trachea. Indian J Surg Oncol. 2016;7:62-66.

54. Green RW, Megwalu UC. Adenoid cystic carcinoma of the external ear: a population based study. Am J Otolaryngol. 2016;37:346-350.

55. Akbaba S, Lang K, Bulut OC, et al. The role of organ- and function-preserving radiotherapy in the treatment of adenoid cystic carcinoma of the larynx. Head Neck. 2019;41:2208-2214.

56. Amit M, Binenbaum Y, Sharma K, et al. Incidence of cervical lymph node metastasis and its association with outcomes in patients with adenoid cystic carcinoma. An international collaborative study. Head Neck. 2015;37:1032-1037.

57. Balamucki CJ, Amdur RJ, Werning JW, et al. Adenoid cystic carcinoma of the head and neck. Am J Otolaryngol. 2012;33:510-518.

58. Xiao R, Sethi RKV, Feng AL, Fontanarosa JB, Deschler DG. The role of elective neck dissection in patients with adenoid cystic carcinoma of the head and neck. Laryngoscope. 2019;129:2094-2104.

59. Suárez C, Barnes L, Silver CE, et al. Cervical lymph node metastasis in adenoid cystic carcinoma of oral cavity and oropharynx: A collective international review. Auris Nasus Larynx. 2016;43:477-484.

60. Garden AS, Weber RS, Ang KK, Morrison WH, Matre J, Peters LJ. Postoperative radiation therapy for malignant tumors of minor salivary glands. Outcome and patterns of failure. Cancer. 1994;73:2563-2569.

61. Garden AS, Weber RS, Morrison WH, Ang KK, Peters LJ. The influence of positive margins and nerve invasion in adenoid cystic carcinoma of the head and neck treated with surgery and radiation. Int J Radiat Oncol Biol Phys. 1995;32:619-626.

62. Thomson DJ, Slevin NJ, Mendenhall WM. Indications for salivary gland radiotherapy. Adv Otorhinolaryngol. 2016;78:141-147.

63. Terhaard CH. Postoperative and primary radiotherapy for salivary gland carcinomas: indications, techniques, and results. Int J Radiat Oncol Biol Phys. 2007;69:S52-5.

64. Gentile MS, Yip D, Liebsch NJ, Adams JA, Busse PM, Chan AW. Definitive proton beam therapy for adenoid cystic carcinoma of the nasopharynx involving the base of skull. Oral Oncol. 2017;65:38-44.

65. Papaspyrou G, Hoch S, Rinaldo A, et al. Chemotherapy and targeted therapy in adenoid cystic carcinoma of the head and neck: a review. Head Neck. 2011;33:905-911.

66. Laurie SA, Ho AL, Fury MG, Sherman E, Pfister DG. Systemic therapy in the management of metastatic or locally recurrent adenoid cystic carcinoma of the salivary glands: a systematic review. Lancet Oncol. 2011;12:815-824.

67. Dodd RL, Slevin NJ. Salivary gland adenoid cystic carcinoma: a review of chemotherapy and molecular therapies. Oral Oncol. 2006;42:759-769.

68. Mücke T, Tannapfel A, Kesting MR, et al. Adenoid cystic carcinomas of minor salivary glands. Auris Nasus Larynx. 2010;37:615-620.

69. Martínez-Rodríguez N, Leco-Berrocal I, Rubio-Alonso L, Arias-Irimia O, Martínez-González JM. Epidemiology and treatment of adenoid cystic carcinoma of the minor salivary glands: a meta-analytic study. Med Oral Patol Oral Cir Bucal. 2011;16:e884-9.

70. Bianchi B, Copelli C, Cocchi R, Ferrari S, Pederneschi N, Sesenna E. Adenoid cystic carcinoma of intraoral minor salivary glands. Oral Oncol. 2008;44:1026-1031.

71. Lukšić I, Suton P, Macan D, Dinjar K. Intraoral adenoid cystic carcinoma: is the presence of perineural invasion associated with the size of the primary tumour, local extension, surgical margins, distant metastases, and outcome? Br J Oral Maxillofac Surg. 2014;52:214-218.

72. He S, Li P, Zhong Q, et al. Clinicopathologic and prognostic factors in adenoid cystic carcinoma of head and neck minor salivary glands: A clinical analysis of 130 cases. Am J Otolaryngol. 2017;38:157-162.

73. Meyers M, Granger B, Herman P, et al. Head and neck adenoid cystic carcinoma: A prospective multicenter REFCOR study of 95 cases. Eur Ann Otorhinolaryngol Head Neck Dis. 2016;133:13-17.

74. Takebayashi S, Shinohara S, Tamaki H, et al. Adenoid cystic carcinoma of the head and neck: a retrospective multicenter study. Acta Otolaryngol. 2018;138:73-79.

75. Amit M, Binenbaum Y, Sharma K, et al. Analysis of failure in patients with adenoid cystic carcinoma of the head and neck. An international collaborative study. Head Neck. 2014;36:998-1004.

76. Marcinow A, Ozer E, Teknos T, et al. Clinicopathologic predictors of recurrence and overall survival in adenoid cystic carcinoma of the head and neck: a single institutional experience at a tertiary care center. Head Neck. 2014;36:1705-1711.

77. Ciccolallo L, Licitra L, Cantú G, Gatta G, EUROCARE Working Group. Survival from salivary glands adenoid cystic carcinoma in European populations. Oral Oncol. 2009;45:669-674.

78. Li N, Xu L, Zhao H, El-Naggar AK, Sturgis EM. A comparison of the demographics, clinical features, and survival of patients with adenoid cystic carcinoma of major and minor salivary glands versus less common sites within the Surveillance, Epidemiology, and End Results registry. Cancer. 2012;118:3945-3953.

79. Spiro RH. Distant metastasis in adenoid cystic carcinoma of salivary origin. Am J Surg. 1997;174:495-498.

80. Luukkaa H, Klemi P, Leivo I, Vahlberg T, Grénman R. Prognostic significance of Ki-67 and p53 as tumor markers in salivary gland malignancies in Finland: an evaluation of 212 cases. Acta Oncol. 2006;45:669-675.

81. Tang QL, Fan S, Li HG, et al. Expression of Cyr61 in primary salivary adenoid cystic carcinoma and its relation to Ki-67 and prognosis. Oral Oncol. 2011;47:365-370.

82. Triantafillidou K, Dimitrakopoulos J, Iordanidis F, Koufogiannis D. Management of adenoid cystic carcinoma of minor salivary glands. J Oral Maxillofac Surg. 2006;64:1114-1120.

83. Gao M, Hao Y, Huang MX, et al. Clinicopathological study of distant metastases of salivary adenoid cystic carcinoma. Int J Oral Maxillofac Surg. 2013;42:923-928.

84. van der Wal JE, Becking AG, Snow GB, van der Waal I. Distant metastases of adenoid cystic carcinoma of the salivary glands and the value of diagnostic examinations during follow-up. Head Neck. 2002;24:779-783.

85. Walker PJ, Siddell SG, Lefkowitz EJ, et al. Changes to virus taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2019). Arch Virol. 2019;164:2417-2429.

86. Moens U, Calvignac-Spencer S, Lauber C, et al. ICTV Virus Taxonomy Profile: Polyomaviridae. J Gen Virol. 2017;98:1159-1160.

87. Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet. 1971;1:1253-1257.

88. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet. 1971;1:1257-1260.

89. Dalianis T, Hirsch HH. Human polyomaviruses in disease and cancer. Virology. 2013;437:63-72.

90. Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. PLoS Pathog. 2009;5:e1000363.

91. Colvin EK, Weir C, Ikin RJ, Hudson AL. SV40 TAg mouse models of cancer. Semin Cell Dev Biol. 2014;27:61-73.

92. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8:e180-e190.

93. Guidry JT, Scott RS. The interaction between human papillomavirus and other viruses. Virus Res. 2017;231:139-147.

94. Cheng J, DeCaprio JA, Fluck MM, Schaffhausen BS. Cellular transformation by Simian virus 40 and murine oolyoma virus T antigens. Semin Cancer Biol. 2009;19:218-228.

95. Polz-Gruszka D, Morshed K, Jarzyński A, Polz-Dacewicz M. Prevalence of Polyoma BK Virus (BKPyV), Epstein-Barr Virus (EBV) and Human Papilloma Virus (HPV) in Oropharyngeal Cancer. Pol J Microbiol. 2015;64:323-328.

96. Kutsuna T, Zheng H, Abdel-Aziz HO, et al. High JC virus load in tongue carcinomas may be a risk factor for tongue tumorigenesis. Virchows Arch. 2008;452:405-410.

97. Zheng Y, Xia P, Zheng HC, Takahashi H, Masuda S, Takano Y. The screening of viral risk factors in tongue and pharyngolaryngeal squamous carcinoma. Anticancer Res. 2010;30:1233-1238.

98. Carpén T, Syrjänen S, Jouhi L, et al. Epstein-Barr virus (EBV) and polyomaviruses are detectable in oropharyngeal cancer and EBV may have prognostic impact. Cancer Immunol Immunother. 2020;69:1615-1626.

99. Monaco MC, Jensen PN, Hou J, Durham LC, Major EO. Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection. J Virol. 1998;72:9918-9923.

100. Silvoniemi A, Mikola E, Ivaska L, et al. Intratonsillar detection of 27 distinct viruses: A cross-sectional study. J Med Virol. 2020;92:3830-3838.

101. Sadeghi M, Aaltonen LM, Hedman L, Chen T, Söderlund-Venermo M, Hedman K. Detection of TS polyomavirus DNA in tonsillar tissues of children and adults: evidence for site of viral latency. J Clin Virol. 2014;59:55-58.

102. Saláková M, Košlabová E, Vojtěchová Z, Tachezy R, Šroller V. Detection of human polyomaviruses MCPyV, HPyV6, and HPyV7 in

malignant and non-malignant tonsillar tissues. J Med Virol. 2016;88:695-702.

103. Herberhold S, Hellmich M, Panning M, et al. Human polyomavirus and human papillomavirus prevalence and viral load in non-malignant tonsillar tissue and tonsillar carcinoma. Med Microbiol Immunol. 2017;206:93-103.

104. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science. 2008;319:1096-1100.

105. Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst. 2009;101:938-945.

106. Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H. Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. Clin Cancer Res. 2011;17:4806-4813.

107. Sihto H, Böhling T, Kavola H, et al. Tumor infiltrating immune cells and outcome of Merkel cell carcinoma: a population-based study. Clin Cancer Res. 2012;18:2872-2881.

108. Chen AA, Gheit T, Stellin M, et al. Oncogenic DNA viruses found in salivary gland tumors. Oral Oncol. 2017;75:106-110.

109. Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. Proc Soc Exp Biol Med. 1953;83:414-421.

110. Stewart SE, Eddy BE, Borgese N. Neoplasms in mice inoculated with a tumor agent carried in tissue culture. J Natl Cancer Inst. 1958;20:1223-1243.

111. Dawe CJ, Freund R, Mandel G, Ballmer-Hofer K, Talmage DA, Benjamin TL. Variations in polyoma virus genotype in relation to tumor induction in mice. Characterization of wild type strains with widely differing tumor profiles. Am J Pathol. 1987;127:243-261.

112. Martinelli M, Martini F, Rinaldi E, et al. Simian virus 40 sequences and expression of the viral large T antigen oncoprotein in human pleomorphic adenomas of parotid glands. Am J Pathol. 2002;161:1127-1133.

113. Burk RD, Chen Z, Van Doorslaer K. Human papillomaviruses: genetic basis of carcinogenicity. Public Health Genomics. 2009;12:281-290.

114. Ndiaye C, Mena M, Alemany L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. Lancet Oncol. 2014;15:1319-1331.

115. Tommasino M. The human papillomavirus family and its role in carcinogenesis. Semin Cancer Biol. 2014;26:13-21.

116. Bishop JA, Yonescu R, Batista D, Yemelyanova A, Ha PK, Westra WH. Mucoepidermoid carcinoma does not harbor transcriptionally active high risk human papillomavirus even in the absence of the MAML2 translocation. Head Neck Pathol. 2014;8:298-302.

117. Bishop JA, Westra WH. Human papillomavirus-related multiphenotypic sinonasal carcinoma: An emerging tumor type with a unique microscopic appearance and a paradoxical clinical behaviour. Oral Oncol. 2018;87:17-20.

118. Burke B. The role of matrix metalloproteinase 7 in innate immunity. Immunobiology. 2004;209:51-56.

119. Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. Oral Dis. 2004;10:311-318.

120. Amar S, Smith L, Fields GB. Matrix metalloproteinase collagenolysis in health and disease. Biochim Biophys Acta Mol Cell Res. 2017;1864:1940-1951.

121. Uitto VJ, Overall CM, McCulloch C. Proteolytic host cell enzymes in gingival crevice fluid. Periodontol 2000. 2003;31:77-104.

122. Luukkaa H, Klemi P, Hirsimäki P, et al. Matrix metalloproteinase (MMP)-7 in salivary gland cancer. Acta Oncol. 2010;49:85-90.

123. Mäkinen LK, Häyry V, Hagström J, et al. Matrix metalloproteinase-7 and matrix metalloproteinase-25 in oral tongue squamous cell carcinoma. Head Neck. 2014;36:1783-1788.

124. López-Boado YS, Wilson CL, Hooper LV, Gordon JI, Hultgren SJ, Parks WC. Bacterial exposure induces and activates matrilysin in mucosal epithelial cells. J Cell Biol. 2000;148:1305-1315.

125. Wilson CL, Matrisian LM. Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions. Int J Biochem Cell Biol. 1996;28:123-136.

126. Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. Amino Acids. 2011;41:271-290.

127. Kuula H, Salo T, Pirilä E, et al. Local and systemic responses in matrix metalloproteinase 8-deficient mice during Porphyromonas gingivalis-induced periodontitis. Infect Immun. 2009;77:850-859.

128. Balbin M, Fueyo A, Tester AM, et al. Loss of collagenase-2 confers increased skin tumor susceptibility to male mice. Nat Genet. 2003;35:252-257.

129. Sorsa T, Salo T, Koivunen E, et al. Activation of type IV procollagenases by human tumor-associated trypsin-2. J Biol Chem. 1997;272:21067-21074.

130. Huang H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors (Basel). 2018;18:10.3390/s18103249.

131. Lemjabbar H, Gosset P, Lamblin C, et al. Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. Am J Respir Crit Care Med. 1999;159:1298-1307.

132. Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res. 1998;58:1048-1051.

133. Wen J, Yin P, Li L, et al. Knockdown of matrix metallopeptidase 9 inhibits metastasis of oral squamous cell carcinoma cells in a zebrafish xenograft model. Biomed Res Int. 2020;2020:4350783.

134. Abraham R, Schäfer J, Rothe M, Bange J, Knyazev P, Ullrich A. Identification of MMP-15 as an anti-apoptotic factor in cancer cells. J Biol Chem. 2005;280:34123-34132.

135. Pei D. Leukolysin/MMP25/MT6-MMP: a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. Cell Res. 1999;9:291-303.

136. Starr AE, Bellac CL, Dufour A, Goebeler V, Overall CM. Biochemical characterization and N-terminomics analysis of leukolysin, the membrane-type 6 matrix metalloprotease (MMP25): chemokine and vimentin cleavages enhance cell migration and macrophage phagocytic activities. J Biol Chem. 2012;287:13382-13395.

137. Luukkaa H, Klemi P, Leivo I, et al. Expression of matrix metalloproteinase-1, -7, -9, -13, Ki-67, and HER-2 in epithelial-myoepithelial salivary gland cancer. Head Neck. 2010;32:1019-1027.

138. de Vicente JC, Lequerica-Fernández P, López-Arranz JS, Esteban I, Fresno MF, Astudillo A. Expression of matrix metalloproteinase-9 in high-grade salivary gland carcinomas is associated with their metastatic potential. Laryngoscope. 2008;118:247-251.

139. Luukkaa H, Klemi P, Hirsimäki P, et al. Matrix metalloproteinase (MMP)-1, -9 and -13 as prognostic factors in salivary gland cancer. Acta Otolaryngol. 2008;128:482-490.

140. Zhao L, Jiang L, Du P, et al. Expression of SKA1 and MMP-9 in primary salivary adenoid cystic carcinoma: Correlation with tumor progression and patient prognosis. Acta Otolaryngol. 2016;136:575-579.

141. Piao S, Zhao S, Guo F, et al. Increased expression of CD147 and MMP-9 is correlated with poor prognosis of salivary duct carcinoma. J Cancer Res Clin Oncol. 2012;138:627-635.

142. Souza Freitas V, Dos Santos JN, de Andrade Santos PP, Nonaka CFW, Pereira Pinto L, de Souza LB. Expression of matrix metalloproteinases (MMPs-2, -7, -9, and -26) and tissue inhibitors of metalloproteinases (TIMPs-1 and -2) in pleomorphic adenomas and adenoid cystic carcinomas. Eur Arch Otorhinolaryngol. 2018;275:3075-3082.

143. Chuang HC, Su CY, Huang HY, et al. Active matrix metalloproteinase-7 is associated with invasion in buccal squamous cell carcinoma. Mod Pathol. 2008;21:1444-1450.

144. Ahmed Haji Omar A, Haglund C, Virolainen S, et al. MMP-7, MMP-8, and MMP-9 in oral and cutaneous squamous cell carcinomas. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119:459-467.

145. Miguel AFP, Mello FW, Melo G, Rivero ERC. Association between immunohistochemical expression of matrix metalloproteinases and metastasis in oral squamous cell carcinoma: Systematic review and meta-analysis. Head Neck. 2020;42:569-584.

146. Korpi JT, Kervinen V, Maklin H, et al. Collagenase-2 (matrix metalloproteinase-8) plays a protective role in tongue cancer. Br J Cancer. 2008;98:766-775.

147. Ruokolainen H, Pääkkö P, Turpeenniemi-Hujanen T. Expression of matrix metalloproteinase-9 in head and neck squamous cell carcinoma: a potential marker for prognosis. Clin Cancer Res. 2004;10:3110-3116.

148. Bodnar M, Szylberg L, Kazmierczak W, Marszalek A. Differentiated expression of membrane type metalloproteinases (MMP-14, MMP-15) and pro-MMP2 in laryngeal squamous cell carcinoma. A novel mechanism. J Oral Pathol Med. 2013;42:267-274.

149. Xie M, Sun Y, Li Y. Expression of matrix metalloproteinases in supraglottic carcinoma and its clinical implication for estimating lymph node metastases. Laryngoscope. 2004;114:2243-2248.

150. Zhang H, Liu M, Sun Y, Lu J. MMP-14 can serve as a prognostic marker in patients with supraglottic cancer. Eur Arch Otorhinolaryngol. 2009;266:1427-1434.

151. Thomas T, Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci. 2001;58:244-258.

152. Kanerva K, Mäkitie LT, Bäck N, Andersson LC. Ornithine decarboxylase antizyme inhibitor 2 regulates intracellular vesicle trafficking. Exp Cell Res. 2010;316:1896-1906.

153. Ramos-Molina B, Lambertos A, Peñafiel R. Antizyme inhibitors in polyamine metabolism and beyond: Physiopathological implications. Med Sci (Basel). 2018;6:89. doi: 10.3390/medsci6040089.

154. Fujita K, Murakami Y, Hayashi S. A macromolecular inhibitor of the antizyme to ornithine decarboxylase. Biochem J. 1982;204:647-652.

155. Kitani T, Fujisawa H. Purification and characterization of antizyme inhibitor of ornithine decarboxylase from rat liver. Biochim Biophys Acta. 1989;991:44-49.

156. Pitkänen LT, Heiskala M, Andersson LC. Expression of a novel human ornithine decarboxylase-like protein in the central nervous system and testes. Biochem Biophys Res Commun. 2001;287:1051-1057.

157. Rasila T, Lehtonen A, Kanerva K, Mäkitie LT, Haglund C, Andersson LC. Expression of ODC antizyme inhibitor 2 (AZIN2) in human secretory cells and tissues. PLoS One. 2016;11:e0151175.

158. Kanerva K, Lappalainen J, Mäkitie LT, Virolainen S, Kovanen PT, Andersson LC. Expression of antizyme inhibitor 2 in mast cells and role of polyamines as selective regulators of serotonin secretion. PLoS One. 2009;4:e6858.

159. Auvinen M, Laine A, Paasinen-Sohns A, et al. Human ornithine decarboxylase-overproducing NIH3T3 cells induce rapidly growing, highly vascularized tumors in nude mice. Cancer Res. 1997;57:3016-3025.

160. Qiu S, Liu J, Xing F. Antizyme inhibitor 1: a potential carcinogenic molecule. Cancer Sci. 2017;108:163-169.

161. Qi L, Chan TH, Tenen DG, Chen L. RNA editome imbalance in hepatocellular carcinoma. Cancer Res. 2014;74:1301-1306.

162. Kaprio T, Rasila T, Hagström J, et al. Ornithine decarboxylase antizyme inhibitor 2 (AZIN2) is a signature of secretory phenotype and independent predictor of adverse prognosis in colorectal cancer. PLoS One. 2019;14:e0211564.

163. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.

164. McNees AL, White ZS, Zanwar P, Vilchez RA, Butel JS. Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. J Clin Virol. 2005;34:52-62.

165. Sorsa T, Ingman T, Suomalainen K, et al. Identification of proteases from periodontopathogenic bacteria as activators of latent human neutrophil and fibroblast-type interstitial collagenases. Infect Immun. 1992;60:4491-4495.

166. Chen JC, Gnepp DR, Bedrossian CW. Adenoid cystic carcinoma of the salivary glands: an immunohistochemical analysis. Oral Surg Oral Med Oral Pathol. 1988;65:316-326.

167. Ellington CL, Goodman M, Kono SA, et al. Adenoid cystic carcinoma of the head and neck: Incidence and survival trends based on 1973-2007 Surveillance, Epidemiology, and End Results data. Cancer. 2012;118:4444-4451.

168. Zhang Y, Liu H, Wu Q, Wang S, Dai C. Predictors of distant metastasis and survival in adenoid cystic carcinoma of the external auditory canal. Otol Neurotol. 2019;40:e1006-e1011.

169. Luksic I, Suton P, Macan D, Dinjar K. Intraoral adenoid cystic carcinoma: is the presence of perineural invasion associated with the size of the primary tumour, local extension, surgical margins, distant metastases, and outcome? Br J Oral Maxillofac Surg. 2014;52:214-218.

170. Marcinow A, Ozer E, Teknos T, et al. Clinicopathologic predictors of recurrence and overall survival in adenoid cystic carcinoma of the head and neck: a single institutional experience at a tertiary care center. Head Neck. 2014;36:1705-1711.

171. Dantas AN, Morais EF, Macedo RA, Tinoco JM, Morais Mde L. Clinicopathological characteristics and perineural invasion in adenoid cystic carcinoma: a systematic review. Braz J Otorhinolaryngol. 2015;81:329-335.

172. Bradley PJ. Adenoid cystic carcinoma evaluation and management: progress with optimism! Curr Opin Otolaryngol Head Neck Surg. 2017;25:147-153.

173. Hirvonen K, Bäck L, Saarilahti K, Leivo I, Hagström J, Mäkitie AA. Pattern of recurrent disease in major salivary gland adenocystic carcinoma. Virchows Arch. 2015;467:19-25.

174. Jeffers LK, Madden V, Webster-Cyriaque J. BK virus has tropism for human salivary gland cells in vitro: implications for transmission. Virology. 2009;394:183-193.

175. Ramqvist T, Ursu RG, Haeggblom L, et al. Human Polyomaviruses are not frequently present in cancer of the salivary glands. Anticancer Res. 2018;38:2871-2874.

176. Sandros J, Stenman G. Karyotypic instability and viral integration in polyoma virus-induced mouse salivary gland tumors. Genes Chromosomes Cancer. 1990;2:109-115.

177. Maginnis MS, Atwood WJ. JC virus: an oncogenic virus in animals and humans? Semin Cancer Biol. 2009;19:261-269.

178. Shen CH, Wu JD, Hsu CD, et al. The high incidence of JC virus infection in urothelial carcinoma tissue in Taiwan. J Med Virol. 2011;83:2191-2199.

179. Eftimov T, Enchev Y, Tsekov I, Simeonov P, Kalvatchev Z, Encheva E. JC polyomavirus in the aetiology and pathophysiology of glial tumours. Neurosurg Rev. 2016;39:47-53.

180. Selgrad M, Koornstra JJ, Fini L, et al. JC virus infection in colorectal neoplasia that develops after liver transplantation. Clin Cancer Res. 2008;14:6717-6721.

181. Uleri E, Piu C, Caocci M, et al. Multiple Signatures of the JC polyomavirus in paired normal and altered colorectal mucosa indicate a link with human colorectal cancer, but not with cancer progression. Int J Mol Sci. 2019;20:5965. doi: 10.3390/ijms20235965.

182. Mifsud M, Eskander A, Irish J, et al. Evolving trends in head and neck cancer epidemiology: Ontario, Canada 1993-2010. Head Neck. 2017;39:1770-1778.

183. Zheng T, Holford TR, Chen Y, et al. Are cancers of the salivary gland increasing? Experience from Connecticut, USA. Int J Epidemiol. 1997;26:264-271.

184. Davies L, Welch HG. Epidemiology of head and neck cancer in the United States. Otolaryngol Head Neck Surg. 2006;135:451-457.

185. Boland JM, McPhail ED, García JJ, Lewis JE, Schembri-Wismayer DJ. Detection of human papilloma virus and p16 expression in high-grade adenoid cystic carcinoma of the head and neck. Mod Pathol. 2012;25:529-536.

186. Qian X, Kaufmann AM, Chen C, et al. Prevalence and associated survival of high-risk HPV-related adenoid cystic carcinoma of the salivary glands. Int J Oncol. 2016;49:803-811.

187. Liu HX, Wu M, Sun YM, Han FY, Liu YF, Zhang GZ. Prognostic value of human papillomavirus infection and p53, p16, epidermal growth factor receptor and p34(cdc2) expression in patients with salivary adenoid cystic carcinoma. Int J Clin Exp Pathol. 2017;10:7882-7889.

188. Hafed L, Farag H, Shaker O, El-Rouby D. Is human papilloma virus associated with salivary gland neoplasms? An *in situ*-hybridization study. Arch Oral Biol. 2012;57:1194-1199.

189. Mohamed FE, Aldayem LN, Hemaida MA, et al. Molecular detection of human papillomavirus-16 among Sudanese patients diagnosed with squamous cell carcinoma and salivary gland carcinoma. BMC Res Notes. 2021 Feb 9;14(1):56.

190. Pujada A, Walter L, Patel A, et al. Matrix metalloproteinase MMP9 maintains epithelial barrier function and preserves mucosal lining in colitis associated cancer. Oncotarget. 2017;8:94650-94665.

191. Kayano K, Shimada T, Shinomiya T, et al. Activation of pro-MMP-2 mediated by MT1-MMP in human salivary gland carcinomas: possible regulation of pro-MMP-2 activation by TIMP-2. J Pathol. 2004;202:403-411.

192. Radunovic M, Nikolic N, Milenkovic S, et al. The MMP-2 and MMP-9 promoter polymorphisms and susceptibility to salivary gland cancer. J BUON. 2016;21:597-602.

193. Kochurova EV. Comparative Role of matrixins in diagnostics of parotid gland tumors. Bull Exp Biol Med. 2019;166:383-385.

194. Jiang WG, Sanders AJ, Katoh M, et al. Tissue invasion and metastasis: Molecular, biological and clinical perspectives. Semin Cancer Biol. 2015;35 Suppl:S244-S275.

195. Liu W, Yang R, Payne AS, et al. Identifying the target cells and mechanisms of Merkel mell polyomavirus infection. Cell Host Microbe. 2016;19:775-787.

196. Iannetta M, Zingaropoli MA, Latronico T, et al. Dynamic changes of MMP-9 plasma levels correlate with JCV reactivation and immune activation in natalizumab-treated multiple sclerosis patients. Sci Rep. 2019 Jan 22;9(1):311.

197. Kaprio T, Rasila T, Hagstrom J, et al. Ornithine decarboxylase antizyme inhibitor 2 (AZIN2) is a signature of secretory phenotype and independent predictor of adverse prognosis in colorectal cancer. PLoS One. 2019;14:e0211564.

198. Shigeyasu K, Okugawa Y, Toden S, et al. AZIN1 RNA editing confers cancer stemness and enhances oncogenic potential in colorectal cancer. JCI Insight. 2018;3:e99976.

199. Rasila T, Lehtonen A, Kanerva K, Makitie LT, Haglund C, Andersson LC. Expression of ODC Antizyme Inhibitor 2 (AZIN2) in Human Secretory Cells and Tissues. PLoS One. 2016;11:e0151175.

200. Kanerva K, Makitie LT, Back N, Andersson LC. Ornithine decarboxylase antizyme inhibitor 2 regulates intracellular vesicle trafficking. Exp Cell Res. 2010;316:1896-1906.

201. Kanerva K, Lappalainen J, Makitie LT, Virolainen S, Kovanen PT, Andersson LC. Expression of antizyme inhibitor 2 in mast cells and role of polyamines as selective regulators of serotonin secretion. PLoS One. 2009;4:e6858.

Karoliina Hirvonen, Adenoid cystic carcinoma of salivary glands: Diagnostic and prognostic factors and treatment outcome. Dissertationes Scolae Doctoralis ad Sanitatem Investigandam universitatis Helsinkiensis 59/2017.